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<p>(21) International Application Number: <b>PCT/US96/08014</b></p> <p>(22) International Filing Date: 30 May 1996 (30.05.96)</p> <p>(30) Priority Data:</p> <table> <tr><td>08/458,423</td><td>2 June 1995 (02.06.95)</td><td>US</td></tr> <tr><td>08/458,424</td><td>2 June 1995 (02.06.95)</td><td>US</td></tr> <tr><td>08/465,591</td><td>5 June 1995 (05.06.95)</td><td>US</td></tr> <tr><td>08/465,594</td><td>5 June 1995 (05.06.95)</td><td>US</td></tr> <tr><td>08/479,725</td><td>7 June 1995 (07.06.95)</td><td>US</td></tr> <tr><td>08/479,783</td><td>7 June 1995 (07.06.95)</td><td>US</td></tr> <tr><td>08/618,693</td><td>20 March 1996 (20.03.96)</td><td>US</td></tr> </table>		08/458,423	2 June 1995 (02.06.95)	US	08/458,424	2 June 1995 (02.06.95)	US	08/465,591	5 June 1995 (05.06.95)	US	08/465,594	5 June 1995 (05.06.95)	US	08/479,725	7 June 1995 (07.06.95)	US	08/479,783	7 June 1995 (07.06.95)	US	08/618,693	20 March 1996 (20.03.96)	US	<p>(71) Applicant (<i>for all designated States except US</i>): NEXSTAR PHARMACEUTICALS, INC. [US/US]; Suite 200, 2860 Wilderness Place, Boulder, CO 80301 (US).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (<i>for US only</i>): TOOTHMAN, Penelope, J. [US/US]; 1880 Sumac Avenue, Boulder, CO 80304 (US). GOLD, Larry [US/US]; 1033 5th Street, Boulder, CO 80303 (US). JANJIC, Nebojsa [US/US]; 6973 Carter Trail, Boulder, CO 80301 (US). RINGQUIST, Steven [US/US]; 221 5th Avenue, Lyons, CO 80540 (US). PAGRATIS, Nikos [US/US]; 5812 N. Orchard Cr. Circle, Boulder, CO 80301 (US).</p> <p>(74) Agents: SWANSON, Barry, J. et al.; Suite 200, 8400 E. Prentice Avenue, Englewood, CO 80111 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>								
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<p>(60) Parent Applications or Grants</p> <p>(63) Related by Continuation</p> <table> <tr><td>US</td><td>08/458,423 (CIP)</td></tr> <tr><td>Filed on</td><td>2 June 1995 (02.06.95)</td></tr> <tr><td>US</td><td>08/458,424 (CIP)</td></tr> <tr><td>Filed on</td><td>2 June 1995 (02.06.95)</td></tr> <tr><td>US</td><td>08/465,591 (CIP)</td></tr> <tr><td>Filed on</td><td>5 June 1995 (05.06.95)</td></tr> <tr><td>US</td><td>08/465,594 (CIP)</td></tr> <tr><td>Filed on</td><td>5 June 1995 (05.06.95)</td></tr> <tr><td>US</td><td>08/479,725 (CIP)</td></tr> <tr><td>Filed on</td><td>7 June 1995 (07.06.95)</td></tr> <tr><td>US</td><td>08/479,783 (CIP)</td></tr> <tr><td>Filed on</td><td>7 June 1995 (07.06.95)</td></tr> <tr><td>US</td><td>08/618,693 (CIP)</td></tr> <tr><td>Filed on</td><td>20 March 1996 (20.03.96)</td></tr> </table>				US	08/458,423 (CIP)	Filed on	2 June 1995 (02.06.95)	US	08/458,424 (CIP)	Filed on	2 June 1995 (02.06.95)	US	08/465,591 (CIP)	Filed on	5 June 1995 (05.06.95)	US	08/465,594 (CIP)	Filed on	5 June 1995 (05.06.95)	US	08/479,725 (CIP)	Filed on	7 June 1995 (07.06.95)	US	08/479,783 (CIP)	Filed on	7 June 1995 (07.06.95)	US	08/618,693 (CIP)	Filed on	20 March 1996 (20.03.96)
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<p>(57) Abstract</p> <p>Methods are described for the identification and preparation of high-affinity nucleic acid ligands to TGF<math>\beta</math>, PDGF and hKGF. Included in the invention are specific RNA and ssDNA ligands to TGF<math>\beta</math>1 and PDGF identified by the SELEX method. Also included in the invention are specific RNA ligands to hKGF identified by the SELEX method. Further included are RNA ligands that inhibit the interaction of TGF<math>\beta</math>1 and hKGF with their receptors and DNA ligands that inhibit the interaction of PDGF with its receptor.</p>																															

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## HIGH AFFINITY OLIGONUCLEOTIDE LIGANDS TO GROWTH FACTORS

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FIELD OF THE INVENTION

Described herein are methods for identifying and preparing high-affinity nucleic acid ligands to TGF $\beta$ , PDGF, and hKGF. The method utilized herein for identifying such nucleic acid ligands is called SELEX, an acronym for Systematic Evolution of Ligands by EXponential enrichment. This invention includes high affinity nucleic acid ligands of TGF $\beta$ , PDGF, and hKGF. Further disclosed are RNA and DNA ligands to TGF $\beta$ 1 and PDGF and RNA ligands to hKGF. Also included are oligonucleotides containing nucleotide derivatives chemically modified at the 2'- positions of pyrimidines. Additionally disclosed are RNA ligands to TGF $\beta$ 1 and hKGF containing 2'-NH<sub>2</sub>-modifications or 2'-F-modifications and RNA ligands to PDGF containing 2'-F modifications. This invention also includes high affinity nucleic acid inhibitors of TGF $\beta$ 1, PDGF, and hKGF. The oligonucleotides of the present invention are useful as pharmaceuticals or diagnostic agents.

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BACKGROUND OF THE INVENTIONTGF $\beta$ 

The transforming growth factor - $\beta$  (TGF $\beta$ ) polypeptides influence growth, differentiation, and gene expression in many cell types. The first polypeptide of this family that was characterized, TGF $\beta$ 1 has two identical 112 amino acid subunits which are covalently linked. TGF $\beta$ 1 is a highly conserved protein with only a single amino acid difference distinguishing human from mice forms. There are two other members of the TGF $\beta$  gene family that are expressed in mammals. TGF $\beta$ 2 is 71% homologous to TGF $\beta$ 1(de Martin *et al.*, (1987) EMBO J. 6:3673-3677), whereas TGF $\beta$ 3 is 80% homologous to TGF $\beta$ 1(Derynck *et al.*, (1988) EMBO J. 7:3737-3743). The structural characteristics of TGF $\beta$ 1 as determined by nuclear magnetic resonance (Archer *et al.*, (1993) Biochemistry

32:1164-1171) agree with the crystal structure of TGF $\beta$ 2 (Daopin *et al.*, (1992) Science 257:369-374; Schlunegger and Grutter (1992) Nature 358:430-434).

Even though the TGF $\beta$ 's have similar three dimensional structures, they are by no means physiologically equivalent. There are at least three different extracellular receptors, type I, II and III, involved in transmembrane signaling of TGF $\beta$  to cells carrying the receptors. For reviews, see Derynck (1994) TIBS 19:548-553 and Massague (1990) Annu. Rev. Cell Biol 6:597-641. In order for TGF $\beta$ 2 to effectively interact with the type II TGF $\beta$  receptor, the type III receptor must also be present (Derynck (1994) TIBS 19:548-553). Vascular endothelial cells lack the type III receptor. Instead endothelial cells express a structurally related protein called endoglin (Cheifetz *et al.*, (1992) J. Biol. Chem. 267:19027-19030), which only binds TGF $\beta$ 1 and TGF $\beta$ 3 with high affinity. Thus, the relative potency of the TGF $\beta$ 's reflect the type of receptors expressed in a cell and organ system.

In addition to the regulation of the components in the multifactorial signaling pathway, the distribution of the synthesis of TGF $\beta$  polypeptides also affects physiological function. The distribution of TGF $\beta$ 2 and TGF $\beta$ 3 is more limited (Derynck *et al.*, (1988) EMBO J 7:3737-3743) than TGF $\beta$ 1, e.g., TGF $\beta$ 3 is limited to tissues of mesenchymal origin, whereas TGF $\beta$ 1 is present in both mesenchymal and epithelial cells.

TGF $\beta$ 1 is a multifunctional cytokine critical for tissue repair. High concentrations of TGF $\beta$ 1 are delivered to the site of injury by platelet granules (Assoian and Sporn, (1986) J Cell Biol. 102:1217-1223.). TGF $\beta$ 1 initiates a series of events that promote healing including chemotaxis of cells such as leukocytes, monocytes and fibroblasts, and regulation of growth factors and cytokines involved in angiogenesis, cell division associated with tissue repair and inflammatory responses. TGF $\beta$ 1 also stimulates the synthesis of extracellular matrix components (Roberts *et al.*, (1986) Proc. Natl. Acad Sci USA 83:4167-4171; Sporn *et al.*, (1983) Science 219:1329-1330; Massague, (1987) Cell 49:437-438) and most importantly for understanding the pathophysiology of

TGF $\beta$ 1, TGF $\beta$ 1 autoregulates its own synthesis (Kim *et al.*, (1989) J Biol Chem 264:7041-7045).

A number of diseases have been associated with TGF $\beta$ 1 overproduction. Fibrotic diseases associated with TGF $\beta$ 1 overproduction can be divided into chronic conditions such as fibrosis of kidney, lung and liver and more acute conditions such as dermal scarring and restenosis. Synthesis and secretion of TGF $\beta$ 1 by tumor cells can also lead to immune suppression such as seen in patients with aggressive brain or breast tumors (Arteaga *et al.*, (1993) J Clin Invest 92: 2569-2576). The course of Leishmanial infection in mice is drastically altered by TGF $\beta$ 1 (Barral-Netto *et al.*, (1992) Science 257:545-547). TGF $\beta$ 1 exacerbated the disease, whereas TGF $\beta$ 1 antibodies halted the progression of the disease in genetically susceptible mice. Genetically resistant mice became susceptible to Leshmanial infection upon administration of TGF $\beta$ 1.

The profound effects of TGF $\beta$ 1 on extracellular matrix deposition have been reviewed (Rocco and Ziyadeh, (1991) in Contemporary Issues in Nephrology v23, Hormones, autocoids and the kidney. ed. Jay Stein, Churchill Livingston, NY pp391-410; Roberts *et al.*, (1988) Rec. Prog. Hormone Res. 44:157-197) and include the stimulation of the synthesis and the inhibition of degradation of extracellular matrix components. Since the structure and filtration properties of the glomerulus are largely determined by the extracellular matrix composition of the mesangium and glomerular membrane, it is not surprising that TGF $\beta$ 1 has profound effects on the kidney. The accumulation of mesangial matrix in proliferative glomerulonephritis (Border *et al.*, (1990) Kidney Int. 37:689-695) and diabetic nephropathy (Mauer *et al.*, (1984) J. Clin Invest. 74:1143-1155) are clear and dominant pathological features of the diseases. TGF $\beta$ 1 levels are elevated in human diabetic glomerulosclerosis (advanced neuropathy) (Yamamoto *et al.*, (1993) Proc. Natl. Acad. Sci. 90:1814-1818). TGF $\beta$ 1 is an important mediator in the genesis of renal fibrosis in a number of animal models (Phan *et al.*, (1990) Kidney Int. 37:426; Okuda *et al.*, (1990) J. Clin. Invest. 86:453). Suppression of experimentally induced glomerulonephritis in rats has been demonstrated by antiserum against TGF $\beta$ 1 (Border *et al.*, (1990) Nature

346:371) and by an extracellular matrix protein, decorin, which can bind TGF $\beta$ 1 (Border *et al.*, (1992) Nature 360:361-363).

Too much TGF $\beta$ 1 leads to dermal scar-tissue formation. Neutralizing TGF $\beta$ 1 antibodies injected into the margins of healing wounds in rats have been shown to inhibit scarring without interfering with the rate of wound healing or the tensile strength of the wound (Shah *et al.*, (1992) Lancet 339:213-214). At the same time there was reduced angiogenesis, reduced number of macrophages and monocytes in the wound, and a reduced amount of disorganized collagen fiber deposition in the scar tissue.

TGF $\beta$ 1 may be a factor in the progressive thickening of the arterial wall which results from the proliferation of smooth muscle cells and deposition of extracellular matrix in the artery after balloon angioplasty. The diameter of the restenosed artery may be reduced 90% by this thickening, and since most of the reduction in diameter is due to extracellular matrix rather than smooth muscle cell bodies, it may be possible to open these vessels to 50% simply by reducing extensive extracellular matrix deposition. In uninjured pig arteries transfected *in vivo* with a TGF $\beta$ 1 gene, TGF $\beta$ 1 gene expression was associated with both extracellular matrix synthesis and hyperplasia (Nabel *et al.*, (1993) Proc. Natl. Acad. Sci USA 90:10759-10763). The TGF $\beta$ 1 induced hyperplasia was not as extensive as that induced with PDGF-BB, but the extracellular matrix was more extensive with TGF $\beta$ 1 transfectants. No extracellular matrix deposition was associated with FGF-1 (a secreted form of FGF) induced hyperplasia in this gene transfer pig model (Nabel (1993) Nature 362:844-846).

There are several types of cancer where TGF $\beta$ 1 produced by the tumor may be deleterious. MATLyLu rat cancer cells (Steiner and Barrack, (1992) Mol. Endocrinol. 6:15-25) and MCF-7 human breast cancer cells (Arteaga *et al.*, (1993) Cell Growth and Differ. 4:193-201) became more tumorigenic and metastatic after transfection with a vector expressing the mouse TGF $\beta$ 1. In breast cancer, poor prognosis is associated with elevated TGF $\beta$  (Dickson *et al.*, (1987) Proc. Natl. Acad. Sci. USA 84:837-841; Kasid *et al.*, (1987) Cancer Res. 47:5733-5738; Daly *et al.*, (1990) J Cell Biochem 43:199-211; Barrett-Lee *et al.*, (1990) Br. J Cancer

61:612-617; King *et al.*, (1989) J Steroid Biochem 34:133-138; Welch *et al.*,  
1990) Proc. Natl. Acad Sci. 87:7678-7682; Walker *et al.*, (1992) Eur J Cancer  
238: 641-644) and induction of TGF $\beta$ 1 by tamoxifen treatment (Butta *et al.*,  
1992) Cancer Res 52:4261-4264) has been associated with failure of tamoxifen  
5 treatment for breast cancer (Thompson *et al.*, (1991) Br. J Cancer 63:609-614).

Anti TGF $\beta$ 1 antibodies inhibit the growth of MDA-231 human breast cancer cells  
in athymic mice (Arteaga *et al.*, (1993) J Clin Invest 92: 2569-2576), a treatment  
which is correlated with an increase in spleen natural killer cell activity. CHO  
cells transfected with latent TGF $\beta$ 1 also showed decreased NK activity and  
10 increased tumor growth in nude mice (Wallick *et al.*, (1990) J Exp Med  
172:1777-1784). Thus, TGF $\beta$ 1 secreted by breast tumors may cause an endocrine  
immune suppression.

High plasma concentrations of TGF $\beta$ 1 have been shown to indicate poor  
prognosis for advanced breast cancer patients (Anscher *et al.*, (1993) N Engl J  
15 Med 328:1592-8). Patients with high circulating TGF $\beta$  before high dose  
chemotherapy and autologous bone marrow transplantation are at high risk for  
hepatic veno-occlusive disease (15-50% of all patients with a mortality rate up to  
50%) and idiopathic interstitial pneumonitis (40-60% of all patients). The  
implication of these findings is 1) that elevated plasma levels of TGF $\beta$ 1 can be  
20 used to identify at risk patients and 2) that reduction of TGF $\beta$ 1 could decrease the  
morbidity and mortality of these common treatments for breast cancer patients.

#### PDGF

Platelet-derived growth factor (PDGF) was originally isolated from  
platelet lysates and identified as the major growth-promoting activity present in  
25 serum but not in plasma. Two homologous PDGF isoforms have been identified,  
PDGF A and B, which are encoded by separate genes (on chromosomes 7 and 22).  
The most abundant species from platelets is the AB heterodimer, although all  
three possible dimers (AA, AB and BB) occur naturally. Following translation,  
30 PDGF dimers are processed into  $\approx$ 30 kDa secreted proteins. Two cell surface  
proteins that bind PDGF with high affinity have been identified,  $\alpha$  and  $\beta$  (Heldin *et*  
*al.*, Proc. Natl. Acad. Sci., 78: 3664 (1981); Williams *et al.*, Proc. Natl. Acad. Sci.,

79: 5867 (1981)). Both species contain five immunoglobulin-like extracellular domains, a single transmembrane domain and an intracellular tyrosine kinase domain separated by a kinase insert domain. The functional high affinity receptor is a dimer and engagement of the extracellular domain of the receptor by PDGF results in cross-phosphorylation (one receptor tyrosine kinase phosphorylates the other in the dimer) of several tyrosine residues. Receptor phosphorylation leads to a cascade of events that results in the transduction of the mitogenic or chemotactic signal to the nucleus. For example, in the intracellular domain of the PDGF  $\beta$  receptor, nine tyrosine residues have been identified that when phosphorylated interact with different src-homology 2 (SH2) domain-containing proteins including phospholipase C-g, phosphatidylinositol 3'-kinase, GTPase-activating protein and several adapter molecules like Shc, Grb2 and Nck (Heldin, Cell, 80: 213 (1995)). In the last several years, the specificities of the three PDGF isoforms for the three receptor dimers (aa, a $\beta$ , and  $\beta\beta$ ) has been elucidated. The a-receptor homodimer binds all three PDGF isoforms with high affinity, the  $\beta$ -receptor homodimer binds only PDGF BB with high affinity and PDGF AB with approximately 10-fold lower affinity, and the a $\beta$ -receptor heterodimer binds PDGF BB and PDGF AB with high affinity (Westerman & Heldin, Acta Oncologica, 32: 101 (1993)). The specificity pattern results from the ability of the A-chain to bind only to the a-receptor and of the B-chain to bind to both a and  $\beta$ -receptor subunits with high affinity.

The earliest indication that PDGF expression is linked to malignant transformation came with the finding that the amino acid sequence of the PDGF-B chain is virtually identical to that of p28<sup>sis</sup>, the transforming protein of the simian sarcoma virus (SSV) (Waterfield *et al.* Nature, 304: 35 (1983); Johnsson *et al.*, EMBO J., 3: 921 (1984)). The transforming potential of the PDGF-B chain gene and, to a lesser extent, the PDGF-A gene was demonstrated soon thereafter (Clarke *et al.*, Nature, 308: 464 (1984); Gazit *et al.*, Cell, 39: 89 (1984); Beckmann *et al.*, Science, 241: 1346; Bywater *et al.*, Mol. Cell. Biol., 8: 2753 (1988)). Many tumor cell lines have since been shown to produce and secrete PDGF, some of which also express PDGF receptors (Raines *et al.*, Peptide Growth

Factors and Their Receptors, Springer-Verlag, Part 1, p 173 (1990)). Paracrine and, in some cell lines, autocrine growth stimulation by PDGF is therefore possible. For example, analysis of biopsies from human gliomas has revealed the existence of two autocrine loops: PDGF-B/β-receptor in tumor-associated endothelial cells and PDGF-A/α-receptor in tumor cells (Hermansson *et al.*, Proc. Natl. Acad. Sci., 85: 7748 (1988); Hermansson *et al.*, Cancer Res., 52: 3213 (1992)). The progression to high grade glioma was accompanied by the increase in expression of PDGF-B and the β-receptor in tumor-associated endothelial cells and PDGF-A in glioma cells. Increased expression of PDGF and/or PDGF receptors has also been observed in other malignancies including fibrosarcoma (Smits *et al.*, Am. J. Pathol., 140: 639 (1992)) and thyroid carcinoma (Heldin *et al.*, Endocrinology, 129: 2187 (1991)).

In view of its importance in proliferative disease states, antagonists of PDGF may find useful clinical applications. Currently, antibodies to PDGF (Johnsson *et al.*, (1985) Proc. Natl. Acad. Sci., U. S. A. 82: 1721-1725; Ferns *et al.*, (1991) Science 153: 1129-1132; Herrenet *et al.*, (1993) Biochimica et Biophysica Acta 1173, 194-302) and the soluble PDGF receptors (Herrenet *et al.*, (1993) Biochimica et Biophysica Acta 1173: 194-302; Duanet *et al.*, (1991) J. Biol. Chem. 266: 413-418; Teisman *et al.*, (1993) J. Biol. Chem. 268: 9621-9628) are the most potent and specific antagonists of PDGF. Neutralizing antibodies to PDGF have been shown to revert the SSV-transformed phenotype (Johnsson *et al.*, (1985) Proc. Natl. Acad. Sci., U. S. A. 82: 1721-1725) and to inhibit the development of neointimal lesions following arterial injury (Ferns *et al.*, (1991) Science 153: 1129-1132). Other inhibitors of PDGF such as suramin (Williams *et al.*, (1984) J. Biol. Chem. 259: 5287-5294; Beisholtz *et al.*, (1984) Cell 39: 447-457), neomycin (Vassbotn *et al.*, (1992) J. Biol. Chem. 267: 15635-15641) and peptides derived from the PDGF amino acid sequence (Engström *et al.*, 1992) J. Biol. Chem. 267: 16581-16587) have been reported, however, they are either too toxic or lack sufficient specificity or potency to be good drug candidates. Other types of antagonists of possible clinical utility are molecules that selectively inhibit the PDGF receptor tyrosine kinase (Buchdunger *et al.*, (1995) Proc. Natl.

Acad. Sci., U. S. A. 92: 2558-2562; Kovalenk *et al.*, (1994) Cancer Res. 54: 6106-6114).

### hKGF

#### *a) Biochemical properties of hKGF*

Human Keratinocyte Growth Factor (hKGF) is a small (26-28KD) basic heparin-binding growth factor and a member of the FGF family. hKGF is a relatively newly identified molecule, which is also known as FGF-7 (Finch *et al.*, (1989) Science 244:752-755). It is a growth factor specific for epithelial cells (Rubin *et al.*, (1989) Proc Natl Acad Sci USA 86:802-806), and its main function is in development/morphogenesis (Werner *et al.*, (1994) Science 266:819-822) and in wound healing (Werner *et al.*, (1992) Proc Natl Acad Sci USA 89:6896-6900). The major *in vivo* source of hKGF is stromal fibroblasts (Finch *et al.*, (1989) Science 244:752-755). Microvascular endothelial cells (Smola *et al.*, (1993) J Cell Biol 122:417-429) and very recently, activated intraepithelial gd T cells (Boismenu *et al.*, (1994) Science 266:1253-1255) have also been shown to synthesize hKGF. hKGF expression is stimulated in wounds (Werner *et al.*, (1992) Proc Natl Acad Sci USA 89:6896-6900). Several cytokines are shown to be hKGF inducers (Brauchle *et al.*, (1994) Oncogene 9:3199-3204), with IL-1 the most potent one (Brauchle *et al.*, (1994) Oncogene 9:3199-3204; Chedid *et al.*, (1994) J Biol Chem 269:10753-10757). Unlike bFGF, hKGF has a signal peptide and thus is secreted by producing cells (Finch *et al.*, (1989) Science 244:752-755). hKGF can be overexpressed in *E. coli* and the recombinant protein (~19-21 KD) is biologically active (Ron *et al.*, (1993) J Biol Chem 268:2984-2988). The *E. coli* derived recombinant protein is 10 times more mitogenic than the native protein (Ron *et al.*, (1993) J Biol Chem 268:2984-2988). This difference may be due to glycosylation. The native protein has a potential Asn glycosylation site (Ron *et al.*, (1993) J Biol Chem 268:2984-2988).

The hKGF bioactivity is mediated through a specific cell surface receptor (Miki *et al.*, (1991) Science 251:72-75). The hKGF receptor is a modified FGF receptor resulting from alternative splicing of the C-terminal extracellular region of the FGF-R2 (Miki *et al.*, (1992) Proc Natl Acad Sci USA 89:246-250).

NIH/3T3 cells transfected with the hKGF receptor express high affinity (~200 pM) binding sites for hKGF (Miki *et al.*, (1992) Proc Natl Acad Sci USA 89:246-250). The approximate number of specific binding sites per NIH/3T3 cell is about 500,000 (D. Bottaro and S. Aaronson, personal communication). The 5 hKGF receptor binds hKGF and aFGF with similar affinities, and bFGF with about 20 fold less affinity (Miki *et al.*, (1991) Science 251:72-75; Miki *et al.*, (1992) Proc Natl Acad Sci USA 89:246-250). A variant of the hKGF receptor has been found to be an amplified gene (i.e., one gene, multiple copies), designated K-SAM, in a human stomach carcinoma cell line (Hattori *et al.*, (1990) 10 Proc natl Acad Sci USA 87: 5983-5987).

Heparin has been reported to be an inhibitor of hKGF bioactivity (Ron *et al.*, (1993) J Biol Chem 268:2984-2988). This is in contrast to the agonistic effect of heparin for aFGF (Spivak-Kroixman *et al.*, (1994) Cell 79:1015-1024).

*b) Role of hKGF in human disease*

15 The recombinant hKGF molecule has been available only since 1993. Therefore, there is limited information on the role of hKGF in human disease. The published literature, however, contains evidence that strongly suggests a role for hKGF in at least two human diseases, namely psoriasis and cancer. hKGF has also been implicated in inflammatory bowel disease (P. Finch, personal 20 communication).

*Psoriasis*

25 Psoriasis is a skin disorder which can be debilitating (Greaves *et al.*, (1995) N Eng J Medicine 332: 581-588), characterized by hyperproliferation of the epidermis and incomplete differentiation of keratinocytes, together with dermal inflammation (Abel *et al.*, (1994) Scientific American Medicine III-1 to III-18; Greaves *et al.*, (1995) N Eng J Medicine 332:581-588). There is not yet an effective treatment for psoriasis (Anonymous, (1993) Drug & Market Development 4:89-101; Abel *et al.*, (1994) Scientific American Medicine III-1 to III-18; Greaves *et al.*, (1995) N Eng J Medicine 332:581-588). Psoriasis occurs in 30 0.5 to 2.8 percent of the population with the highest incidence in Scandinavia. In the US in 1992, it was estimated that 4-8 million people affected with psoriasis

spent about \$600 million for various drugs and related therapies, none of which is very effective. Most of the expenditure was made by about 400,000 patients with severe psoriasis spending \$1,000-1,500 annually on treatment. There are about 200,000 new cases of psoriasis every year.

5       The basic cause of the disorder is not known, but it results from a primary or secondary defect in the mechanisms that regulate epidermal keratinocyte cell division (Abel *et al.*, (1994) Scientific American Medicine III-1 to III-18). Psoriasis responds to steroids and cyclosporine and in that sense is characterized as an immune disease (Abel *et al.*, (1994) Scientific American Medicine III-1 to III-18). Since hKGF is the primary specific growth factor for keratinocytes, its overexpression and deregulation are primary candidates as the cause of keratinocyte hyperproliferation in psoriasis. The demonstration that the immune system is a prime regulator of hKGF release (Boismenu *et al.*, (1994) Science 266: 1253-1255; Brauchle *et al.*, (1994) Oncogene 9: 3199-3204; Chedid *et al.*, (1994) J Biol Chem 269: 10753-10757) strengthens the notion that hKGF deregulation is the cause of psoriasis. Furthermore, application of hKGF in porcine wounds creates a histological appearance resembling psoriasis (Staiano-Coico *et al.*, (1993) J Ex Med 178:865-878); keratinocyte derived hKGF in transgenic mice causes pathology reminiscent to psoriasis (Guo *et al.*, (1993) EMBO J 12: 973-986); *in situ* hybridization experiments demonstrated a moderate and a strong upregulation of hKGF and hKGF receptors respectively in psoriasis (P. Finch, personal communication). *In situ* hybridization experiments also demonstrated involvement of hKGF in another immune disease namely, inflammatory bowel disease (P. Finch, personal communication).

20      **Cancer**

25      It is well established in the literature that deregulation of the expression of growth factors and growth factor hKGF and/or its receptor is expected to be the transformation event in some human cancers. The transforming ability of the hKGF system has been demonstrated *in vitro* (Miki *et al.*, (1991) Science 251:72-75). In another study, carcinoma cell-lines have been found to express the hKGF receptor and to respond to hKGF but not to aFGF, while sarcoma cell-lines

do not express hKGF receptors and respond to aFGF but not to hKGF (Ishii *et al.*, (1994) Cancer Res **54**:518-522).

#### ***Gastrointestinal Cancer***

Several poorly differentiated stomach cancers have an amplified gene, designated K-sam, which is an isoform of the hKGF-receptor (Katoh *et al.*, (1992) Proc Natl Acad Sci USA **89**:2960-2964). *In vivo* administration of hKGF to rats causes proliferation of pancreatic ductal epithelial cell (Yi *et al.*, (1994) Am J Pathol **145**:80-85), hepatocytes, and epithelial cells throughout the gastrointestinal tract (Housley *Et al.*, (1994) J Clin Invest **94**:1764-1777).

#### ***Lung Cancer***

Administration of hKGF to rats causes type II pneumocyte hyperplasia similar to the bronchoalveolar cell variant of lung carcinoma (Ulich *et al.*, (1994) J Clin Invest **93**:1298-1306).

#### ***Breast Cancer***

*In vivo*, hKGF causes mammary duct dilation and rampant epithelial hyperplasia, both of which are common features of breast cancers (Ulich *et al.*, (1994) Am J Pathol **144**:862-868; Yi *et al.*, (1994) Am J Pathol **145**:1015-1022). However, the ductal epithelium of breastfeeding rats is resistant to the growth promoting effects of hKGF and this is of interest in regard to epidemiological observations that pregnancy in women decreases susceptibility to breast cancer and that dairy cows almost never develop breast cancer (Kuzma, 1977, *Breast in Pathology*, Mosby Co.). There is additional supporting evidence implicating hKGF in breast cancer. hKGF mRNA has been detected recently in normal human breast tissue and in 12 of 15 breast tumor samples tested (Koos *et al.*, (1993) J Steroid Biochem Molec Biol **45**:217-225). The presence of hKGF mRNA in breast tumors considered in conjunction with the observation that hKGF is present in nonneoplastic mammary glands and that hKGF causes rampant proliferation of mammary epithelium suggests that hKGF may be an autocrine or paracrine growth factor important in the regulation of the growth of normal and neoplastic mammary epithelium (Ulich *et al.*, (1994) Am J Pathol **144**:862-868). Infiltrating ductal mammary adenocarcinoma is characteristically enveloped by a

desmoplastic stroma that has been postulated to represent a defensive host response to the carcinoma (Ulich *et al.*, (1994) Am J Pathol **144**:862-868). Since hKGF is stroma derived it is possible that the desmoplastic stroma contributes rather than inhibits the growth of the tumor.

5

#### ***Prostate Cancer***

The growth promoting effect of androgens on prostate tumors appears to be mediated through hKGF (Yan *et al.*, (1992) Mol Endo **6**:2123-2128), as androgens induce the expression of hKGF in prostate stroma cells. Prostate tumors that are androgen dependent *in vivo*, are androgen independent *in vitro*, but hKGF dependent (Yan *et al.*, (1992) Mol Endo **6**:2123-2128). In agreement with the role of hKGF as andromedin is the observation that hKGF functions in epithelial induction during seminal vesicle development, a process that is directed by androgen (Alarid *et al.*, (1994) Proc Natl Acad Sci USA **91**:1074-1078). Furthermore, hKGF causes aberrant activation of the androgen receptor, thus probably contributing to the failure of androgen ablation therapy in prostate cancer (Culig *et al.*, (1994) Cancer Res **54**:5474-5478). Based on this information, it is possible that genetic alterations cause hKGF to escape androgen regulation and thus convert the androgen dependent tumor into an androgen independent, highly malignant tumor. Such tumors would still be able to express the androgen regulated marker PSA, as hKGF also causes the aberrant activation of the androgen receptor. It is also likely that hKGF might be responsible for Benign Prostate Hypertrophy (BPH), a common health problem in older men (D. Bottaro, personal communication).

#### ***d) hKGF Competitors***

25

To date, a monoclonal antibody and a short hKGF-receptor derived peptide (25-mer) have been described as hKGF competitors (Bottaro *et al.*, (1993) J Biol Chem **268**:9180-9183). The monoclonal antibody, designated 1G4, has a Kd of 200pM for hKGF. The short peptide inhibits hKGF binding to the cell surface of NIH/3T3 cells expressing the human receptor with a Ki of about 1-5  $\mu$ M. Bottaro *et al.* (WO 94/25057) provide hKGF-receptor peptides which inhibit binding

between hKGF and its receptor. Also provided is a method of assaying test compounds for the ability to inhibit hKGF receptor-mediated cell proliferation.

*e) Assaying for receptor-growth factor interaction*

Blocking the interaction of growth factors and lymphokines with their cell surface receptor using antagonists has been an approach for disease treatment. The discovery of such antagonists requires the availability of biochemical assays for the receptor-growth factor or lymphokine interaction. A classic assay has been the competitive inhibition of radiolabeled growth factor or lymphokine (tracer) to its cell surface receptor. These types of assays utilize cell lines that express the relevant receptor on their surface and determines the amount of cell bound tracer in the presence of various concentrations of potential antagonists. Additionally, other assays utilize membrane extracts from cell lines that express the relevant receptor, and tracer binding is followed by filter binding (see Nenquest Drug Discovery System: Human Tumor Necrosis Factor-Alpha, NEN Research Products, E. I. DePont de Nemours & Co. (Inc.), Boston, MA) or by immobilizing the membrane extracts onto solid supports (Urdal *et al.*, (1988) *J Biol Chem* 263:2870-2877; Smith *et al.*, (1991) *Bioch Bioph Res Comm* 176:335-342). Receptor induced electrophoretic mobility shift of tracer has been applied to identify the presence and size of cell surface receptors by crosslinking the receptor to the tracer and then analyzing on denaturing gels (for example see Kull *et al.*, (1985) *Proc natl Acad Sci USA* 82:5756-5760; Hohmann *et al.*, (1989) *J Biol Chem* 264:14927-14934; Stauber *et al.*, (1989) *J Biol Chem* 264:3573-3576). The use of native gels and non-crosslinked complexes has not been described for growth factors or lymphokines and their receptors, but has been widely applied to study nucleic acid protein interactions (Sambrook *et al.*, (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Screening of various cancer cell lines for the presence of hKGF receptors by PCR, revealed that all carcinoma cell lines express hKGF receptor mRNA while sarcoma cell lines do not. The presence of mRNA does not necessarily mean that hKGF receptor will be present on the surface of these cells. For hKGF,

only cell based assays have been described using Balb/MK keratinocytes (Weissman, (1983) *Cell* 32: 599-606) or NIH/3T3 cells transfected with the hKGF receptor (Miki, (1992) *Proc. Natl. Acad. Sci. USA* 89:246-250 ).

**SELEX**

5           A method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules has been developed. This method, Systematic Evolution of Ligands by EXponential enrichment, termed SELEX, is described in United States Patent Application Serial No. 07/536,428, entitled "Systematic Evolution of Ligands by Exponential Enrichment," now abandoned, United States  
10          Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled "Nucleic Acid Ligands," now U.S. Patent No. 5,475,096, United States Patent Application Serial No. 07/931,473, filed August 17, 1992, entitled "Nucleic Acid Ligands," now United States Patent No. 5,270,163 (see also PCT/US91/04078), each of which is herein specifically incorporated by reference. Each of these applications,  
15          collectively referred to herein as the SELEX Patent Applications, describes a fundamentally novel method for making a nucleic acid ligand to any desired target molecule.

20          The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding, partitioning unbound nucleic acids from those  
25          nucleic acids which have bound specifically to target molecules, dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic  
30          acid ligands to the target molecule.

The basic SELEX method has been modified to achieve a number of specific objectives. For example, United States Patent Application Serial No. 07/960,093, filed October 14, 1992, entitled "Method for Selecting Nucleic Acids on the Basis of Structure," describes the use of SELEX in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. United States Patent Application Serial No. 08/123,935, filed September 17, 1993, entitled "Photoselection of Nucleic Acid Ligands" describes a SELEX based method for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoactivating a target molecule. United States Patent Application Serial No. 08/134,028, filed October 7, 1993, entitled "High-Affinity Nucleic Acid Ligands That Discriminate Between Theophylline and Caffeine," describes a method for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed "Counter-SELEX." United States Patent Application Serial No. 08/143,564, filed October 25, 1993, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Solution SELEX," describes a SELEX-based method which achieves highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. United States Patent Application Serial No. 07/964,624, filed October 21, 1992, entitled "Methods of Producing Nucleic Acid Ligands" describes methods for obtaining improved nucleic acid ligands after SELEX has been performed. United States Patent Application Serial No. 08/400,440, filed March 8, 1995, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Chemi-SELEX," describes methods for covalently linking a ligand to its target.

The SELEX method encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX-identified nucleic acid ligands containing modified nucleotides are described in United States Patent Application Serial No. 08/117,991, filed

September 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. United States Patent Application Serial No. 08/134,028, *supra*, describes highly specific 5 nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH<sub>2</sub>), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). United States Patent Application Serial No. 08/264,029, filed June 22, 1994, entitled "Novel Method of Preparation of 2' Modified Pyrimidine Intramolecular Nucleophilic Displacement," describes oligonucleotides containing various 2'-modified 10 pyrimidines.

The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in United States Patent Application Serial No. 08/284,063, filed August 2, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: 15 Chimeric SELEX" and United States Patent Application Serial No. 08/234,997, filed April 28, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Blended SELEX," respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable 20 properties of other molecules. Each of the above described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

#### BRIEF SUMMARY OF THE INVENTION

25 The present invention includes methods of identifying and producing nucleic acid ligands to transforming growth factor beta (TGF $\beta$ ), platelet-derived growth factor (PDGF), and human keratinocyte growth factor (hKGF), and homologous proteins, and the nucleic acid ligands so identified and produced. For the purpose of this application, TGF $\beta$  includes human TGF $\beta$ 1, TGF $\beta$ 2, and 30 TGF $\beta$ 3 and TGF $\beta$ 's that are substantially homologous thereto. By substantially homologous it is meant a degree of amino acid sequence identity of 70% or more.

For the purposes of this application, PDGF refers to PDGF AA, AB, and BB isoforms and homologous proteins. Specifically included in the definition are human PDGF AA, AB and BB isoforms. In particular, RNA sequences are provided that are capable of binding specifically to TGF $\beta$ 1, PDGF, and hKGF.

5       Also provided are ssDNA sequences that are capable of binding specifically to TGF $\beta$  and PDGF. Specifically included in the invention are the RNA ligand sequences shown in Tables 3, 13, 16, and 23 (SEQ ID NOS:12-42, 128-170, 189-262, 272-304). The RNA ligand sequences of TGF $\beta$  shown in Table 3 include both pre and post SELEX modifications. Also included in the invention are  
10      ssDNA ligands of TGF $\beta$  and PDGF shown in Tables 6, 8, 9, and Figures 3, 4, and 9 (SEQ ID NOS:55-89, 93-124, 171-176). Also included in this invention are RNA ligands of TGF $\beta$ 1 and hKGF that inhibit the function of TGF $\beta$ 1 and hKGF, presumably by inhibition of the interaction of TGF $\beta$  and hKGF with their  
15      receptors. Also included in this invention are ssDNA ligands of PDGF that inhibit the function of PDGF, presumably by inhibition of the interaction of PDGF with its receptor.

Further included in this invention is a method of identifying nucleic acid ligands and nucleic acid ligand sequences to a target selected from the group consisting of TGF $\beta$ , PDGF, and hKGF comprising the steps of (a) contacting a  
20      candidate mixture of nucleic acids with the target (b) partitioning between members of said candidate mixture on the basis of affinity to the target and (c) amplifying the selected molecules to yield a mixture of nucleic acids enriched for nucleic acid sequences with a relatively higher affinity for binding to the target.

More specifically, the present invention includes the RNA and ssDNA  
25      ligands to TGF $\beta$  identified according to the above-described method, including those ligands shown in Tables 3 and 6 (SEQ ID NOS:12-42, 55-89). Also included are nucleic acid ligands to TGF $\beta$  that are substantially homologous to any of the given ligands and that have substantially the same ability to bind TGF $\beta$  and inhibit the function of TGF $\beta$ . Further included in this invention are nucleic  
30      acid ligands to TGF $\beta$  that have substantially the same structural form as the

ligands presented herein and that have substantially the same ability to bind TGF $\beta$  and inhibit the function of TGF $\beta$ .

Additionally, the present invention includes the ssDNA and RNA ligands to PDGF identified according to the above-described method, including those 5 ligands shown in Tables 8 and 13, and Figures 3, 4, and 9 (SEQ ID NOS:93-124, 128-176). Also included are DNA and RNA ligands to PDGF that are substantially homologous to any of the given ligands and that have substantially 10 the same ability to bind PDGF. Further included in this invention are nucleic acid ligands to PDGF that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind PDGF.

In addition, the present invention includes the RNA ligands to hKGF identified according to the above-described method, including those ligands 15 shown in Tables 16 and 23 (SEQ ID NOS:189-262, 272-304). Also included are RNA ligands to hKGF that are substantially homologous to any of the given ligands and that have substantially the same ability to bind hKGF and inhibit the interaction of hKGF with its receptor. Further included in this invention are 20 nucleic acid ligands to hKGF that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind hKGF and inhibit the interaction of hKGF with its receptor.

The present invention also includes other modified nucleotide sequences based on the RNA ligands identified herein and mixtures of the same.

Further included in this invention is a method of assaying a test compound 25 for the ability to inhibit hKGF receptor-mediated cell proliferation comprising the steps of (a) contacting the test compound with a hKGF nucleic acid ligand and a keratinocyte growth factor; and (b) detecting the ability of the test compound to inhibit binding between the hKGF nucleic acid ligand and the keratinocyte growth factor.

Also included in this invention is a method of assaying a test compound 30 for the ability to inhibit the interaction of a growth factor with its plasma membrane bound receptor comprising the steps of (a) solubilizing cells containing

the plasma membrane bound receptor; (b) creating a plasma membrane extract of the cells; (c) reacting the extract with labeled growth factor alone and in the presence of the test compound thereby creating complexes; (d) analyzing the complexes by electrophoresis under native conditions; (e) visualizing the complexes by imaging; and (f) comparing the image of the extract with labeled growth factor alone to the image of the extract in the presence of the test compound to determine whether the test compound inhibited the interaction between the growth factor and its plasma membrane bound receptor.

Further included in this invention is a method for assaying cells to determine whether they express a growth factor plasma membrane bound receptor comprising the steps of (a) solubilizing the cells; (b) creating a plasma membrane extract of the cells; (c) reacting the plasma membrane extract with a labeled growth factor; (d) analyzing the reaction between the plasma membrane extract with the labeled growth factor by electrophoresis under native conditions; (e) comparing the electrophoresis of step (d) with electrophoresis of labeled growth factor; and (e) visualizing the results of the electrophoresis to determine whether a complex is formed with altered mobility relative to the mobility of a labeled growth factor alone.

20

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the binding analysis of the 40D7 DNA library for TGF $\beta$ 1. Binding data obtained from Round 19 (triangles) and Round 0 (circles) are shown.

Figure 2 shows the results of the PAI-luciferase assay of TGF $\beta$ 1 (10pM) incubated with oligonucleotides (0.1 $\mu$ M) or anti-TGF $\beta$  (60  $\mu$ g/ml).

Figure 3 shows the consensus secondary structure for the sequence set shown in Table 9. R = A or G, Y = C or T, K = G or T, N and N' indicate any base pair.

Figure 4 shows the minimal ligands 20t, 36t and 41t folded according to the consensus secondary structure motif. [3'T] represents a 3'-3' linked thymidine nucleotide added to reduce 3'-exonuclease degradation.

Figures 5A, 5B and 5C show the binding of minimal high affinity DNA ligands to PDGF-AA, AB and BB, respectively. The fraction of  $^{32}\text{P}$  5' end-labeled DNA ligands bound to varying concentrations of PDGF was determined by the nitrocellulose filter binding method. Minimal ligands tested were 20t (o), 36t ( $\Delta$ ), and 41t (■). Oligonucleotide concentrations in these experiments were  $\approx 10 \text{ pM}$  (PDGF-AB and PDGF-BB) and  $\approx 50 \text{ pM}$  (PDGF AA). Data points were fitted to eq. 1 (for binding of the DNA ligands to PDGF-AA) or to eq. 2 (for binding to PDGF AB and BB) using the non-linear least squares method. Binding reactions were done at  $37^\circ\text{C}$  in binding buffer (PBSM with 0.01% HSA).

Figure 6 shows the dissociation rate determination for the high affinity interaction between the minimal DNA ligands and PDGF AB. The fraction of 5'  $^{32}\text{P}$  end-labeled ligands 20t (o), 36t ( $\Delta$ ), and 41t (■), all at 0.17 nM, bound to PDGF AB (1 nM) was measured by nitrocellulose filter binding at the indicated time points following the addition of a 500-fold excess of the unlabeled competitor. The dissociation rate constant ( $k_{\text{off}}$ ) values were determined by fitting the data points to eq 3. The experiments were performed at  $37^\circ\text{C}$  in binding buffer.

Figure 7 shows the effect of DNA ligands on the binding of  $^{125}\text{I}$ -PDGF-BB and  $^{125}\text{I}$ -PDGF-AA to PDGF  $\alpha$ -receptors expressed in PAE cells.

Figure 8 shows the effect of DNA ligands on the mitogenic effect of PDGF-BB on PAE cells expressing the PDGF  $\beta$ -receptors.

Figure 9 shows the 2'-O-methyl-2'-deoxy- and 2'-fluoro-2'-deoxyribonucleotide-substitution pattern compatible with high affinity binding to PDGF-AB. Underlined symbols indicate 2'-O-methyl-2'-deoxynucleotides; italicized symbols indicate 2'-fluoro-2'-deoxynucleotides; normal font indicates 2'-deoxyribonucleotides; [3'T] indicates inverted orientation (3'3') thymidine nucleotide (Glen Research, Sterling, VA); PEG in the loops of helices II and III indicates pentaethylene glycol spacer phosphoramidite (Glen Research, Sterling, VA).

Figure 10A shows the saturation binding of radiolabeled hKGF on the surface of the PC-3 cells. TB (total binding) is the binding observed in the

absence of competing unlabeled hKGF, whereas NSB (nonspecific binding) is the binding observed in the presence of 100 fold molar excess of unlabeled hKGF. SB (specific binding) demonstrates the specific binding, and this curve was derived by subtracting the NSB curve from the TB curve. Figure 10B is the Scatchard analysis of the data points shown in 10A for the SB curve.

Figure 11 shows the shift of the electrophoretic mobility due to plasma membrane extracts from PC-3 cells. In lanes 1-8, the membrane extracts were reacted with various concentrations of radiolabeled hKGF as shown under each lane. In addition to the radiolabeled hKGF (as shown under each lane) for lanes 9-12 a 100 fold molar excess was included of unlabeled hKGF. C1 and C2 represent two observed complexes due to the presence of hKGF binding moieties in the PC-3 plasma membrane extracts.

Figures 12A - D show the proposed alignment of 2'F and 2'NH<sub>2</sub> ligands. Lower case, italicized sequence residues indicate the constant region of the template. In the consensus sequences, capital and lower case letters are used for residues found in greater than or equal to 80% and 60% of the members of each family respectively.  $K_d$  and  $K_i$  values are also shown next to the designation of each ligand. The  $K_i$  values shown here were calculated using the formula  $K_i = IC50/(1+(C/K_d))$ , where IC50 is the measured half maximal inhibitory concentration of each ligand in the PC-3 cell assay as described in Example 16; C is the concentration of <sup>125</sup>I-KGF; and  $K_d$  is the equilibrium dissociation constant of KGF for its receptor, (about 150 pM). The ligands marked with stars show biphasic binding curves.

Figures 12A and 12B show the proposed alignment of 2'F ligands. The majority of 2'F ligands can be folded into pseudoknot structures. Two classes are proposed as shown. The summary structure for each class is also shown. Bases participating in stem 1 (S1) are underlined with single lines while bases of stem 2 (S2) are underlined with double lines. Spaces were introduced for alignment of the various elements of the pseudoknots.

Figures 12C and 12D show the proposed folding of 2'NH<sub>2</sub> ligands. These ligands are assigned into two classes. As shown in the summary structures, class

1 and class 2 ligands can form a stem-loop and dumbbell structure, respectively. Spaces were introduced to allow sequence alignment. Residues participating in stems are underlined. In the summary structures, periods (.) indicate a variable number of residues. Ligands 2N and 54N are circular permutations of the same 5 dumbbell structure. For alignment of the corresponding loops these ligands are wrapped around two lines.

Figure 13 shows the minimal sequence requirement for binding of ligand 6F and 14F to hKGF. The predicted folding of each ligand is shown. Constant regions of the ligands are shown in lower case. Conserved sequences are 10 underlined. Circles and triangles mark the 3' ends of active and inactive truncates respectively.

#### DETAILED DESCRIPTION OF THE INVENTION

This application describes high-affinity nucleic acid ligands to TGF $\beta$ , 15 PDGF, and hKGF identified through the method known as SELEX. SELEX is described in U.S. Patent Application Serial No. 07/536,428, entitled Systematic Evolution of Ligands by EXponential Enrichment, now abandoned, U.S. Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled Nucleic Acid Ligands, United States Patent Application Serial No. 07/931,473, filed August 17, 20 1992, entitled Nucleic Acid Ligands, now United States Patent No. 5,270,163, (see also PCT/US91/04078). These applications, each specifically incorporated herein by reference, are collectively called the SELEX Patent Applications.

In its most basic form, the SELEX process may be defined by the following series of steps:

- 25 1) A candidate mixture of nucleic acids of differing sequence is prepared. The candidate mixture generally includes regions of fixed sequences (i.e., each of the members of the candidate mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are selected either: (a) to assist in the amplification steps described below, (b) to mimic a sequence known to bind to the target, or (c) to enhance the concentration 30 of a given structural arrangement of the nucleic acids in the candidate mixture.

The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (e.g., the probability of finding a base at any location can be selected at any level between 0 and 100 percent).

5        2) The candidate mixture is contacted with the selected target under conditions favorable for binding between the target and members of the candidate mixture. Under these circumstances, the interaction between the target and the nucleic acids of the candidate mixture can be considered as forming nucleic acid-target pairs between the target and those nucleic acids having the strongest 10 affinity for the target.

15        3) The nucleic acids with the highest affinity for the target are partitioned from those nucleic acids with lesser affinity to the target. Because only an extremely small number of sequences (and possibly only one molecule of nucleic acid) corresponding to the highest affinity nucleic acids exist in the candidate mixture, it is generally desirable to set the partitioning criteria so that a significant amount of the nucleic acids in the candidate mixture (approximately 5-50%) are retained during partitioning.

20        4) Those nucleic acids selected during partitioning as having the relatively higher affinity to the target are then amplified to create a new candidate mixture that is enriched in nucleic acids having a relatively higher affinity for the target.

25        5) By repeating the partitioning and amplifying steps above, the newly formed candidate mixture contains fewer and fewer weakly binding sequences, and the average degree of affinity of the nucleic acids to the target will generally increase. Taken to its extreme, the SELEX process will yield a candidate mixture containing one or a small number of unique nucleic acids representing those nucleic acids from the original candidate mixture having the highest affinity to the target molecule.

30        The SELEX Patent Applications describe and elaborate on this process in great detail. Included are targets that can be used in the process; methods for partitioning nucleic acids within a candidate mixture; and methods for amplifying partitioned nucleic acids to generate enriched candidate mixture. The SELEX

Patent Applications also describe ligands obtained to a number of target species, including both protein targets where the protein is and is not a nucleic acid binding protein.

The nucleic acid ligands described herein can be complexed with a  
5 lipophilic compound (e.g., cholesterol) or attached to or encapsulated in a complex comprised of lipophilic components (e.g., a liposome). The complexed nucleic acid ligands can enhance the cellular uptake of the nucleic acid ligands by a cell for delivery of the nucleic acid ligands to an intracellular target. U.S. Patent Application No. 08/434,465, filed May 4, 1995, entitled "Nucleic Acid Ligand Complexes," which is incorporated in its entirety herein, describes a method for preparing a therapeutic or diagnostic complex comprised of a nucleic acid ligand and a lipophilic compound or a non-immunogenic, high molecular weight compound.

10 The methods described herein and the nucleic acid ligands identified by such methods are useful for both therapeutic and diagnostic purposes. Therapeutic uses include the treatment or prevention of diseases or medical conditions in human patients. Diagnostic utilization may include both *in vivo* or *in vitro* diagnostic applications. The SELEX method generally, and the specific adaptations of the SELEX method taught and claimed herein specifically, are particularly suited for diagnostic applications. SELEX identifies nucleic acid 15 ligands that are able to bind targets with high affinity and with surprising specificity. These characteristics are, of course, the desired properties one skilled in the art would seek in a diagnostic ligand.

20 The nucleic acid ligands of the present invention may be routinely adapted for diagnostic purposes according to any number of techniques employed by those skilled in the art. Diagnostic agents need only be able to allow the user to identify the presence of a given target at a particular locale or concentration. Simply the ability to form binding pairs with the target may be sufficient to trigger a positive signal for diagnostic purposes. Those skilled in the art would also be able to adapt 25 any nucleic acid ligand by procedures known in the art to incorporate a labeling tag in order to track the presence of such ligand. Such a tag could be used in a

number of diagnostic procedures. The nucleic acid ligands described herein may specifically be used for identification of the TGF $\beta$ , PDGF, and hKGF proteins.

SELEX provides high affinity ligands of a target molecule. This represents a singular achievement that is unprecedented in the field of nucleic acids research. The present invention applies the SELEX procedure to the specific targets of TGF $\beta$ , PDGF, and hKGF. In the Example section below, the experimental parameters used to isolate and identify the nucleic acid ligands to TGF $\beta$ , PDGF, and hKGF are described.

In order to produce nucleic acids desirable for use as a pharmaceutical, it is preferred that the nucleic acid ligand (1) binds to the target in a manner capable of achieving the desired effect on the target; (2) be as small as possible to obtain the desired effect; (3) be as stable as possible; and (4) be a specific ligand to the chosen target. In most situations, it is preferred that the nucleic acid ligand have the highest possible affinity to the target.

In co-pending and commonly assigned U.S. Patent Application Serial No. 07/964,624, filed October 21, 1992 ('624), now U.S. Patent No. 5,496,938, methods are described for obtaining improved nucleic acid ligands after SELEX has been performed. The '624 application, entitled Methods of Producing Nucleic Acid Ligands, is specifically incorporated herein by reference. Further included in this patent are methods for determining the three-dimensional structures of nucleic acid ligands. Such methods include mathematical modeling and structure modifications of the SELEX-derived ligands, such as chemical modification and nucleotide substitution.

In the present invention, SELEX experiments were performed in order to identify RNA and DNA ligands with specific high affinity for TGF $\beta$ 1 from degenerate libraries containing 40 or 60 random positions (40N or 60N) (Tables 1 and 5). This invention includes the specific RNA ligands to TGF $\beta$ 1 shown in Table 3 (SEQ ID NOS:12-42), identified by the methods described in Examples 1 and 2. This invention further includes RNA ligands to TGF $\beta$  which inhibit TGF $\beta$ 1 function, presumably by inhibiting the interaction of TGF $\beta$ 1 with its receptor. This invention includes the specific ssDNA ligands to TGF $\beta$ 1 shown in

Table 6 (SEQ ID NOS:55-89) identified by the methods described in Examples 5 and 6.

In the present invention, two SELEX experiments were also performed in order to identify ssDNA and RNA with specific high affinity for PDGF from degenerate libraries containing 40 and 50 random positions (40N and 50N), respectively (Tables 7 and 12). This invention includes the specific ssDNA and RNA ligands to PDGF shown in Tables 8, 9 and 13 and Figures 3, 4, and 9 (SEQ ID NOS:93-124, 128-176 ), identified by the methods described in Examples 7 and 15.

In the present invention, a SELEX experiment was also performed in search of RNA ligands with specific high affinity for hKGF from degenerate libraries containing 40 random positions (40N) (Table 14). This invention includes the specific RNA ligands to hKGF shown in Tables 16 and 23 (SEQ ID NOS:189-262, 272-304), identified by the methods described in Examples 16 and 17. This invention further includes RNA ligands to hKGF which inhibit the interaction of hKGF with its receptor.

The scope of the ligands covered by this invention extends to all nucleic acid ligands of TGF $\beta$ , PDGF, and hKGF, modified and unmodified, preferably those identified according to the SELEX procedure. More specifically, this invention includes nucleic acid sequences that are substantially homologous to the ligands shown in Tables 3, 6, 8, 9, 13, 16, and 23 and Figures 3, 4, and 9 (SEQ ID NOS:12-42, 55-89, 93-124, 128-176, 189-262, 272-304). By substantially homologous it is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%. A review of the sequence homologies of the nucleic acid ligands shown in Tables 3 and 6 (SEQ ID NOS.:12-42, 55-89) for TGF $\beta$ , Tables 8 and 13 (SEQ ID NOS:93-124, 128-170) for PDGF, and Tables 16 and 23 (SEQ ID NOS:189-262, 272-304) for hKGF shows that sequences with little or no primary homology may have substantially the same ability to bind a given target. For these reasons, this invention also includes nucleic acid ligands that have substantially the same structure and ability to bind TGF $\beta$ , PDGF, and hKGF as the nucleic acid ligands shown in Tables 3, 6, 8, 9, 13, 16, and 23 and

Figures 3, 4, and 9 (SEQ ID NOS:12-42, 55-89, 93-124, 128-176, 189-262, 272-304). Substantially the same structure for PDGF includes all nucleic acid ligands having the common structural elements shown in Figure 3 that lead to the affinity to PDGF. Substantially the same ability to bind TGF $\beta$ , PDGF, or hKGF means that the affinity is within one or two orders of magnitude of the affinity of the ligands described herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence -- substantially homologous to those specifically described herein -- has substantially the same ability to bind TGF $\beta$ , PDGF, or hKGF.

This invention also includes the ligands as described above, wherein certain chemical modifications are made in order to increase the *in vivo* stability of the ligand or to enhance or mediate the delivery of the ligand. Examples of such modifications include chemical substitutions at the sugar and/ or phosphate and/or base positions of a given nucleic acid sequence. See, e.g., U.S. Patent Application Serial No. 08/117,991, filed September 9, 1993, entitled High Affinity Nucleic Acid Ligands Containing Modified Nucleotides which is specifically incorporated herein by reference. Other modifications are known to one of ordinary skill in the art. Such modifications may be made post-SELEX (modification of previously identified modified or unmodified ligands) or by incorporation into the SELEX process.

Example 20 describes post-SELEX procedure modification of a nucleic acid ligand to basic fibroblast growth factor (bFGF). The nucleic acid ligand was modified by the addition of phosphorothioate caps and substitution of several ribopurines with 2'-deoxy-2'-O-methylpurines.

As described above, because of their ability to selectively bind TGF $\beta$ , PDGF, and hKGF, the nucleic acid ligands to TGF $\beta$ , PDGF, and hKGF described herein are useful as pharmaceuticals. This invention, therefore, also includes a method for treating TGF- $\beta$ -mediated pathological conditions by administration of a nucleic acid ligand capable of binding to TGF $\beta$ , a method for treating PDGF-mediated pathological conditions by administration of a nucleic acid ligand capable of binding to PDGF, and a method for treating hKGF-mediated

pathological conditions by administration of a nucleic acid ligand capable of binding to hKGF.

Therapeutic compositions of the nucleic acid ligands may be administered parenterally by injection, although other effective administration forms, such as  
5 intraarticular injection, inhalant mists, orally active formulations, transdermal iontophoresis or suppositories, are also envisioned. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment, it is  
10 envisioned that the carrier and the ligand constitute a physiologically-compatible, slow release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other  
15 pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the ligand. Such excipients are those substances usually and customarily employed to formulate dosages for parental administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in  
20 sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or requiring reconstitution immediately prior to administration. The manner of administering formulations containing nucleic acid ligands for systemic delivery may be via subcutaneous, intramuscular, intravenous, intranasal or  
25 vaginal or rectal suppository.

The following examples are provided to explain and illustrate the present invention and are not intended to be limiting of the invention. Examples 1-4 describe initial experiments to identify RNA with specific high affinity for TGF $\beta$ 1. Example 1 describes the various materials and experimental procedures  
30 used in Examples 2-4. Example 2 describes a representative method for identifying RNA ligands by the SELEX method which bind TGF $\beta$ 1. Example 3

describes the affinities the ligands have for TGF $\beta$ 1 and demonstrates that the ligands are capable of inhibiting the function of TGF $\beta$ 1, presumably by inhibiting the interaction of TGF $\beta$ 1 with its receptor. Example 4 describes which regions of the ligands are believed to be necessary for TGF $\beta$ 1 binding and inhibition of TGF $\beta$ 1 receptor binding. Example 5 describes another representative method for identifying RNA and DNA ligands by the SELEX method which bind TGF $\beta$ 1. Example 6 reports on the binding analysis, bioassay, and sequences of a ssDNA SELEX library. Example 7 describes the various materials and experimental procedures used in evolving ssDNA ligands to PDGF described in Examples 8-13.

Example 8 describes the ssDNA ligands to PDGF and the predicted secondary structure of selected nucleic acid ligands. Example 9 describes the minimal sequence necessary for high affinity binding. Example 10 describes the kinetic stability of PDGF-Nucleic Acid Ligand complexes. Example 11 describes the thermal melting properties for selected ligands. Example 12 describes photo-crosslinking of nucleic acid ligands and PDGF. Example 13 describes the inhibition by DNA ligands of PDGF isoforms on cultured cells and inhibition of the mitogenic effects of PDGF in cells by DNA ligands. Example 14 describes the modification of nucleic acid ligands to PDGF with modified nucleotides. Example 15 describes the experimental procedures used in evolving RNA ligands to PDGF and shows the ligand sequences. Example 16 describes the various materials and experimental procedures used in evolving nucleic acid ligands to hKGF described in Examples 17-19. Example 17 describes the RNA ligands to hKGF, the affinities the ligands have for hKGF, and the specificity of the RNA ligands to hKGF. Example 18 describes inhibition of hKGF binding to cell surface receptors. Example 19 reports on the inhibition of mitogenic activity of hKGF by a selected ligand. Example 20 describes the modification of nucleic acid ligands to bFGF with 2'-deoxy-2'-O-methylpurines.

EXAMPLESEXAMPLE 1. EXPERIMENTAL PROCEDURES

This example provides the general procedures followed and incorporated in Examples 2-4.

5       A. Materials.

Human recombinant TGF $\beta$ 1 used in this SELEX procedure was acquired from Genentech. Human recombinant TGF $\beta$ 1 can also be purchased from R&D systems, Minneapolis, MN, USA.

Biotinylated TGF $\beta$ 1 was prepared by reacting TGF $\beta$ 1 at 3.6  $\mu$ M with an 11 fold molar excess of sulfo-NHS-biotin (Pierce, Rockford, IL, USA) in 50mM NaHCO<sub>3</sub> for 3 hr. in an ice bath. The reaction was acidified with 0.036 volumes of 10% acetic acid and applied to a 40 mg. Vydac (The Separations Group, Hesperia, CA, USA) reverse phase column made in a siliconized pipet tip to separate unreacted biotin from biotinylated TGF $\beta$ 1. The column was prewashed with 200  $\mu$ l ethanol followed by 200  $\mu$ l 1% acetic acid, the biotinylation reaction was applied, free biotin was washed through with 200  $\mu$ l of 50 mM sodium acetate pH 5.5, followed by 200  $\mu$ l of 20% acetonitrile and finally eluted with 200  $\mu$ l of 60% acetonitrile. The sample was lyophilized and resuspended in 50 mM sodium acetate pH 5.0 at 40  $\mu$ M and stored at 4° C. The TGF $\beta$ 1 was spiked with 100,000 cpm iodinated TGF $\beta$ 1 in order to follow recovery and to assess the success of the biotinylation reaction by measuring the fraction of the radioactivity that would bind to streptavidin coated agarose beads (Pierce) before and after biotinylation. An aliquot of the TGF $\beta$ 1 before and after biotinylation was subjected to analytical reverse phase chromatography. The biotinylated TGF $\beta$ 1 substantially ran as a single peak which was retarded with respect to the unbiotinylated TGF $\beta$ . A small amount (5 %) of unreacted TGF $\beta$ 1 could be detected. The efficiency of binding of the iodinated, biotinylated TGF $\beta$ 1 to streptavidin (SA) agarose beads (30 $\mu$ l) was 30 % under the binding conditions used for SELEX partitioning.

Iodinated TGF $\beta$ 1 was prepared by the lactoperoxidase method (50mM 30       sodium phosphate pH 7.3, 0.16% glucose) with BioRad Enzymo beads (BioRad,

Richmond, CA, USA) and the bound iodine separated from the free iodine by gel filtration on G25 Sephadex in 50 mM sodium acetate 0.01% Tween.

The mink lung cell line expressing the luciferase reporter gene under the control of PAI 1 promoter (Abe *et al.* (1994) *Anal. Biochem.* 216:276-284) was a gift from Dr. Dan Rifkin (Department of Cell Biology, New York Medical Center, New York, New York 10016). Luciferase was assayed by reagents purchased from Analytical Luminescence Laboratory, San Diego, CA, USA.

2'- NH<sub>2</sub> modified CTP and UTP were prepared according to the method of Pieken *et al.* (1991) *Science* 253:314-317. DNA oligonucleotides were synthesized using standard procedures either at NeXstar Pharmaceuticals, Inc. (Boulder, CO, USA) or by Operon Technologies (Alameda, CA, USA). All other reagents and chemicals were purchased from standard commercial sources and sources have been indicated.

#### **B. SELEX procedure**

SELEX ligands that bind to TGF $\beta$ 1 were derived essentially as described in U.S. Patent No. 5,270,163 (*see also*, Tuerk and Gold (1990) *Science* 249:505-510). To generate the starting pool of PCR template, PCR product from twenty separate PCR reactions each containing 16.1 pmol of unpurified, single stranded DNA (at least a total of  $2 \times 10^{12}$  to  $2 \times 10^{13}$  different molecules) were

pooled before the first transcription. PCR conditions were 50 mM KCl, 10 mM Tris-HCL, pH 9, 0.1% Triton-X100, 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dATP, dCTP, dGTP, and dTTP, 2 $\mu$ M each primer and 0.075 units/ $\mu$ l of Taq DNA polymerase, 100  $\mu$ l per reaction in a siliconized microfuge tube. All PCR cycles took advantage of hot start using Ampliwax (Perk and Elmer, Norwalk, CN, USA).

Duration of the initial PCR was 10 cycles; a PCR cycle was 94° C-1', 52° C-1', 72° C-2'. An initial denaturation was 94° C for 4' and the final extension at 72° C for 5'. PCR reactions were combined, phenol/ chloroform extracted, and isopropanol precipitated (2.0 M ammonium acetate, 50% isopropanol) to remove primers.

Transcription reactions contained 200 nM DNA, 0.9 mM GTP, 0.9 mM 2'-NH<sub>2</sub>-UTP, 0.9 mM 2'-NH<sub>2</sub>-CTP, 0.5mM ATP, 87 mM Tris-HCl pH 8.0, 17 mM

MgCl<sub>2</sub>, 4.4 mM spermidine, 22 mM DTT, 100 µg/ml acetylated BSA (Promega, Madison, WI, USA) and 4 units/µl T7 RNA polymerase. ( 2'-F-UTP and 2'-F-CTP (United States Biochemical, Cleveland, OH, USA) were used at 3.0 mM, whereas UTP and CTP were used at 0.9mM each). Transcription reactions were incubated overnight at 28° C (at least 10 hours). After transcription the template was digested by addition of 2µl RQ1 Dnase (Promega) for 15' at 28° C, and then extracted with phenol/CHCl<sub>3</sub>, followed by three ethanol precipitations from ammonium acetate (3.9M ammonium acetate, 72% ethanol).

5

The RNA molecules were incubated with TGFβ1 bound to SA agarose beads as described below in Krebs-Ringer solution (KR) (120 mM NaCl, 4.8 mM KCl, 10 mM Na phosphate buffer pH 7.4, 1.2 mM MgSO<sub>4</sub>, 2.6 mM CaCl<sub>2</sub>) modified to include 20mM Na-Hepes pH 7.5 and 0.2% Triton X100 (Pierce). This buffer is referred to as KRHT.

10

TGFβ1-RNA complexes were separated from unbound RNA by washing the beads. Recovery of the selected 2'-NH<sub>2</sub> or F pyrimidine modified RNA from the agarose beads required guanidine thiocyanate extraction (5M GnSCN, 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.0, 0.1M beta mercaptoethanol) or from Seradyne SA coated beads by 2% SDS (0.1 M Tris-HCl pH 7.5, 50 mM NaCl, 1 mM Na<sub>2</sub>EDTA, 2% SDS, 1.5mM DTT). Regular 2'-OH RNA was easily recovered under less harsh conditions with the same buffer used for the Seradyne beads containing only 0.2% SDS. After extraction and precipitation to purify and concentrate the RNA, the sample was reverse transcribed with a cloned MMLV RT with the RNase H sequence deleted. The reaction contained less than or equal to 16 nM RNA, 10 µM 3' primer, 50 mM Tris-HCL pH 8.3, 75 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM DTT, 0.5 mM dNTP's. Prior to addition of buffer the RNA and the primer were boiled together. After addition of buffer and salts the reaction was annealed for 10 min at 28° C before addition of 600 units of Superscript reverse transcriptase (Bethesda Research Labs, Gaithersburg, MD, USA) and synthesis at 50° C for 1 hour.

PCR amplification of this cDNA (<1 pmol) resulted in approximately 250 pmol double-stranded DNA, of this, 40pmols was transcribed and used to initiate the next round of SELEX.

**C. Partitioning Method for SELEX.**

5        2.5 pmols biotinylated TGF $\beta$ 1 were bound to 30  $\mu$ l SA agarose beads (Pierce) in 200  $\mu$ l KRHT. The mixture was incubated on a rotator at 37°C for 15 to 30 minutes. The beads were washed three times by centrifugation and resuspension in 200  $\mu$ l cold KRHT to remove unbound TGF $\beta$ 1, and resuspended in a final volume of 500  $\mu$ l KRHT. RNA containing 2'-NH<sub>2</sub> pyrimidines was  
10      heated at 70° C for three minutes (RNAs containing 2'-OH or 2'-F pyrimidines were heated at 95° C) and diluted into KRHT containing TGF $\beta$ 1 bound to SA beads. The final concentration of RNA is 1  $\mu$ M and the TGF $\beta$ 1 was 5nM. Binding occurs with rotation at 37° C for 30 minutes. Beads were washed by centrifugation and resuspension three times with 200  $\mu$ l binding buffer to remove  
15      unbound RNA. RNA was eluted from the beads as described above.

**D. Binding assays.**

20      Two binding assays for ligands to TGF $\beta$ 1 gave equivalent results wherever tested. In the SA bead assay the biotinylated TGF $\beta$ 1 was serially diluted in KRHT in polypropylene tubes (Linbro, JCN, Irvine, CA, USA) and bound to the beads as described above. After unbound TGF $\beta$ 1 was washed away, trace quantities of <sup>32</sup>P-labeled RNA(<0.1 nM) were added to each tube and vortexed to mix. After static incubation at 37°C for 30 minutes, the unbound RNA was removed by washing three times with 200  $\mu$ l of KRHT.

25      In the nitrocellulose filter binding assay, TGF $\beta$ 1 was serially diluted in KRH containing 0.1% defatted BSA (Fluka radioimmunoassay grade, Fluka, Hauppauge, NY, USA) as carrier instead of Triton X-100. Incubation with RNA tracer was as above. Samples were pipetted with a multiwell pipettor onto a multiwell manifold holding a sheet of wet BioRad 0.45 micron nitrocellulose, aspirated, and washed three times with 200  $\mu$ l KRH (containing no BSA). The filters were air dried and counted in a liquid scintillation counter (Beckmann Instruments, Palo Alto, CA)

The equation used to fit the binding of ligands to TGF $\beta$ 1 describes the binding of a ligand to a receptor (in this case TGF $\beta$ 1) that follows the laws of mass action and for which there is a single binding site:  $Y=B_{max} \cdot X / (K_d + X)$ : where  $Y$  is the fraction of the ligand bound,  $B_{max}$  is the maximum fraction of the ligand bound,  $X$  is the concentration of TGF $\beta$ 1 and  $K_d$  is the dissociation constant of TGF $\beta$ 1 and the ligand. Data points were fit by nonlinear regression using the computer program Graphpad Prism (Graphpad Software, San Diego, CA). The algorithm minimized the sum of the squares of the actual distance of the points from the curve. Convergence was reached when two consecutive iterations changed the sum-of-squares by less than 0.01%.

#### E. Cloning and Sequencing.

SELEX experiments are described in Table 2. Primers for SELEX experiments 1 and 2 shown in Table 1 contain recognition sites for the restriction endonucleases SacI (5' primer T7SacBam; SEQ ID NO:7) and XbaI (3' primer 3XH; SEQ ID NO:9). PCR products from SELEX experiments 1 and 2 were cloned directionally into SacI/XbaI digested pGem 9zf (Promega). 5' primer T7SB2N (SEQ ID NO:8) and 3' primer 3XH (SEQ ID NO:9) (Table 1) were used for SELEX experiments 3-9. PCR products from SELEX experiments 3-9 were cloned directionally into the BamH1/XbaI site of a modified pGem9zf :BamH1 cloning vector. The BamH1 site was engineered into pGem9zf in the following way. A clone isolated from library 2 (lib2-6-2) that did not bind to TGF $\beta$ 1 (sequence not shown) was digested with BamH1 and XbaI. The sequence flanking the cloning site of the modified pGem9zf vector is shown in Table 1 (SEQ ID NOS:10-11).

After digestion of the plasmid with restriction endonuclease and dephosphorylation with CIP (calf intestinal phosphatase), vectors were gel purified. Inserts were ligated and recombinant plasmids were transformed into *E. coli* strain DH10B (Bethesda Research Labs). Plasmid DNA was prepared by alkaline lysis, mini prep procedure. Twenty-two clones representing 9 unique sequences were sequenced at random from libraries 1 and 2. 50 clones were sequenced from libraries 3-9 using a single dideoxy G reaction (called G track).

The sequencing ladders were compared and organized for similarities. Selected clones from each family were chosen for complete sequence analysis. TGF $\beta$ 1 binding assays were performed on transcripts representing different G sequences in each library. Out of a total of 140 binding assays, 27 ligands bound with a Kd less than 10 nM, and 21 of these were sequenced. Clones were sequenced with the Sequenase sequencing kit (United States Biochemical Corporation, Cleveland, OH).

#### F. Ligand Truncation.

Truncation experiments were carried out to determine the minimal sequence necessary for high affinity binding of the RNA ligands to TGF $\beta$ 1. For 3' boundary determination, RNA ligands were 5' end-labeled with  $\gamma$ - $^{32}$ P-ATP using T4 polynucleotide kinase. 5' boundaries were established with 3' end-labeled ligands using  $\alpha$ - $^{32}$ P-pCp and T4 RNA ligase. After partial alkaline hydrolysis, radiolabeled RNA ligands were incubated with TGF $\beta$ 1 at concentrations ranging from 1 nM to 50 nM and protein-bound RNA was separated by nitrocellulose partitioning. RNA truncates were analyzed on a high-resolution denaturing polyacrylamide gel. A ladder of radioactively labeled ligands terminating with G-residues was generated by partial RNase T1 digestion and was used as markers.

#### G. Inhibition of TGF $\beta$ 1 function.

TGF $\beta$ 1 signal transduction begins with binding to a cell surface receptor and results in the induction of transcription of a variety of genes. One of these genes is Pai1. The TGF $\beta$ 1 assay utilizes the mink lung epithelial cell (MLEC) carrying the luciferase reporter gene fused to the Pai 1 promoter. The MLEC has TGF $\beta$ 1 receptors on its cell surface. Thus one can measure the response of the cells to TGF $\beta$ 1 and the effective concentration of TGF $\beta$ 1 in the culture media by measuring the luciferase enzyme activity after a period of induction.

Mink lung epithelial cells (MLEC) carrying the Pai1/luc construct were maintained in DME containing 10% fetal bovine serum and 400  $\mu$ g/ml G418. MLEC-Pai1/luc cells were plated at  $3.2 \times 10^4$  cells per well in a 96 well Falcon plate, in 100  $\mu$ l of DME + 10% fetal bovine serum overnight. Media was made from autoclaved water. The cells were washed three times (100  $\mu$ l) in serum free

DME plus Solution A (1:1). Solution A is 30 mM Hepes pH 7.6, 10 mM glucose, 3.0 mM KCl, 131 mM NaCl, 1.0 mM disodium phosphate. Samples (100 $\mu$ l) were added in DME containing 20 mM Hepes pH 7.5, and 0.1% BSA (Fluka, radioimmunoassay grade). All samples were in triplicate. After six hours at 37° C  
5 in a 5% CO<sub>2</sub> incubator the media was removed and cells were washed three times (100 $\mu$ l each) in cold PBS. Lysis buffer (75  $\mu$ l) (Analytical Luminescence Laboratory) was added and the plates incubated on ice for 20 min. The plates were sealed and frozen at -80° C until assayed. Samples (25 $\mu$ l) were assayed for  
10 luciferase activity with the Enhanced Luciferase Assay Kit from Analytical Luminescence Laboratory (San Diego, CA, USA) according to the manufacturers instructions using the Berthold Microlumat LB96P luminometer. Luminescence is reproducibly a function of TGF $\beta$ 1 concentration added to the media.

Ligands tested for inhibition of TGF $\beta$ 1 activity were tested at a minimum of five concentrations. The ligands were serially diluted in DME, 20 mM Hepes pH 7.5, 0.1% Fluka BSA in polypropylene tubes and an equal volume of media containing 12 pM TGF $\beta$ 1 was added to each tube, vortexed and transferred to the cells without further incubation. From the TGF $\beta$ 1 standard curve included on every plate the effective concentration of TGF $\beta$ 1 in the presence of the inhibitory ligands was determined by the reduction in luminescence measured. Some  
15 ligands were tested at both 3 pM and 6 pM TGF $\beta$ 1 with the same results. To determine the IC<sub>50</sub> (the concentration of SELEX ligand necessary to reduce the TGF $\beta$ 1 activity 50%), the five values obtained for each ligand were plotted and the value at 50% inhibition was determined graphically using Graphpad Prism assuming a hyperbolic fit of the data and using non-linear regression.  
20

25

#### EXAMPLE 2. RNA LIGANDS TO TGF $\beta$ 1

##### A. SELEX experiments

In order to generate RNA ligands to TGF $\beta$ 1, nine SELEX experiments, as summarized in Table 2, were performed using the methods described in Example  
30 1. As shown in Table 1, the RNA pools differ in the number of random bases present in the central portion of the molecules: 40 nucleotides in the 40N6 (SEQ

ID NO:2) SELEX and 64 nucleotides in the 64N6 and lib2-6-1RN6 (SEQ ID NOS:1, 3) SELEX experiment. Since the goal was to select RNA ligands that not only bound to TGF $\beta$ 1 but also blocked receptor binding, the large random region (64N) was chosen. In two experiments, a shorter random region (40N) was also included. Ligands to TGF $\beta$ 1 were very rare with 40N and were qualitatively the same as the 64N6 ligands selected.

The sequences of clones from the SELEX experiments are shown in Table 3 (SEQ ID NOS:12-42). The pyrimidines used in the various SELEX experiments differed at the 2' position of the sugar (Table 2). In the first two

10 SELEX experiments, ligands were evolved as 2'-OH pyrimidines. Ligands were post-SELEX modified with 2'-NH<sub>2</sub> or 2'-F- substituted pyrimidines to see if they retained TGF $\beta$ 1 binding. Since the 2' substitutions rendered the ligands resistant to RNase A they were also tested in the cell culture assay for inhibition of TGF $\beta$ 1 activity. One such ligand lib2-6-1 (Group D, Table 3; SEQ ID NO:35) when

15 substituted with 2'-NH<sub>2</sub>-UTP and 2'-F-CTP was shown to inhibit TGF $\beta$ 1 receptor mediated activity. To select more ligands, six more independent SELEX experiments (lib3-7 and lib9) were performed using the 2'-F and 2'-NH<sub>2</sub>

-substituted pyrimidines during the evolution process. In experiment lib8 the biologically active clone lib2-6-1 (SEQ ID NO:35) was randomized and subjected

20 to re-selection to see if the binding and inhibition behavior of the clone could be improved. Lib8 was evolved as a 2'-OH pyrimidine RNA. In some cases, post-SELEX modification of TGF $\beta$ 1 ligands derived from experiments 3-9 were performed, e.g., to determine if a ligand evolved with 2'-F pyrimidine substitutions would also bind with 2'-NH<sub>2</sub> substitutions.

25 Each starting pool for a SELEX experiment contained  $3 \times 10^{14}$  RNA molecules (500 pmol). The affinity of the starting RNA for TGF $\beta$ 1 was estimated to be greater than 50 mM. After 4 rounds of SELEX, the affinities of the evolving pools had improved to approximately 10 nM and did not shift significantly in subsequent rounds. RNA was bulk sequenced and found to be non-random and cloned.

Lib1 took 20 rounds to evolve since optimum concentrations of TGF $\beta$ 1 were not used until round 15 and libraries 5, 6 and 7 took longer to evolve because optimum conditions for recovery of bound ligands during the partitioning step in SELEX were not introduced until round 8. Optimum TGF $\beta$ 1 concentrations and partitioning conditions are described in Example 1.

**B. RNA Sequences**

Many clones in a SELEX library are identical or similar in sequence. The libraries were screened by G track and only representatives of each G track type were tested in a binding assay. The binding assay was five points (16.5nM, 10 5.5nM, 1.8nM, 0.6nM, and 0.2nM) and could detect only those SELEX clones with a Kd less than or equal to 10 nM. RNA ligands that bound well (Kd<10nM) in the binding assay were sequenced. The sequences were inspected by eye and analyzed using computer programs which perform alignments and fold nucleic acid sequences to predict regions of secondary structure. Ligands were classified into five groups (A, B, C, D, and orphans) by sequence homology. Each group has characteristic allowable 2' substitutions.

58 clones were identified by G track from 7 separate SELEX experiments to belong to group A ligands (Table 3; SEQ ID NOS:12-42). 15 clones were sequenced; 13 were similar but not identical, whereas 3 clones, lib3-13 (SEQ ID NO:12), lib5-6 and lib5-13, were identical. Group A ligands were recovered from seven of the eight SELEX libraries which included libraries evolved as 2'-NH<sub>2</sub>, 2'-OH or 2'-F -substituted pyrimidines as well as a library evolved as 2'-F-UTP, 2'-NH<sub>2</sub>-CTP. Post SELEX modification indicates that 2'-NH<sub>2</sub>-UTP, 2'-F-CTP does not disrupt binding of lib8-9 to TGF $\beta$ 1, thus the structure of Group A ligands appears to not require a specific 2' moiety on the pyrimidine sugar in order to maintain binding.

Group B ligands bind both as 2'-NH<sub>2</sub> and 2'-F pyrimidine substituted RNA. 28 Group B clones were detected by G track analysis from 3 libraries. Two of the libraries were evolved as 2'-NH<sub>2</sub> and one as 2'-F library. Four clones were sequenced, two were identical (lib5-47 and lib4-12; SEQ ID NO:28). An internal deletion can occur in group B, as in lib 3-44. The 40N orphan, lib3-42 was placed

in Group B on the basis of secondary structure. The internal deletion in lib3-44, the binding affinity, the bioactivity and boundary experiments all support the placement of lib3-42 in this group.

Group C ligands bind as 2'-OH or 2'-F ligands as expected, since members of this group were evolved as 2'-OH ligands in lib 1 and as 2'-F pyrimidine substituted ligands in lib 6. Lib1-20-3 (SEQ ID NO:32) was post SELEX modified and as 2'-F derivative. Lib1-20-3 did not bind with 2'-NH<sub>2</sub> pyrimidines incorporated.

Group D ligand, lib2-6-1 (SEQ ID NO:35), was isolated after 2'-OH SELEX but was post SELEX modified and binds well as a 2'-NH<sub>2</sub>-UTP and 2'-F-CTP pyrimidine derivative. Lib2-6-1 does not bind well to TGF $\beta$ 1 with 2'-NH<sub>2</sub>, 2'-F or 2'F-UTP, 2'-NH<sub>2</sub>-CTP-substituted pyrimidines. Variants of Group D were only reselected in two other SELEX experiments, lib8, a 2'-OH library, and lib 9, a 2'-NH<sub>2</sub>-UTP, 2'-F CTP library, supporting the observation that there is specificity for the 2' pyrimidine position in this ligand.

The group labeled orphans are not homologous to each other and no variant sequences for these ligands have been determined. G track indicates that eight 40N clones similar to lib3-45 were isolated from two libraries. Two of the eight were sequenced and are identical. Lib3-45 (SEQ ID NO:39) binds whether it contains 2'-NH<sub>2</sub> or 2'-F substituted pyrimidines or the 2'-F-UTP, 2'-NH<sub>2</sub>-CTP combination. Lib1-20-5 (SEQ ID NO:40) isolated as a 2'-OH ligand binds as a 2'-F, whereas lib1-20-12 (SEQ ID NO:41) and lib2-6-8 (SEQ ID NO:42) bind well only as 2'-OH pyrimidines and will not tolerate 2'-NH<sub>2</sub> or 2'-F post SELEX modifications.

As it was unusual that similar sequences were obtained from different SELEX experiments containing different modifications, another set of SELEX experiments was performed in search of RNA and ssDNA ligands to TGF $\beta$ 1 as described in examples 5 and 6 *infra*.

EXAMPLE 3. INHIBITION OF TGF $\beta$ 1 RECEPTOR BINDING

The  $K_d$ s and  $B_{max}$  values reported in Table 4 for Group A ligands are for the 2'-NH<sub>2</sub> substituted version of the ligand unless otherwise noted.  $B_{max}$  for the Group A ligands was  $0.38 \pm 0.12$  ( $n=14$ ) which is in agreement with the measured retention of TGF $\beta$ 1 on the nitrocellulose filters. The  $K_d$ 's for Group A ligands were all similar,  $2.2 \pm 1.1$  nM ( $n=14$ ). Where measured nitrocellulose and SA agarose bead binding assays gave equivalent results.

The  $IC_{50}$ 's in Table 4 for Group A ligands were all tested with the 2'-NH<sub>2</sub> pyrimidine substituted ligands except where indicated. 2'-NH<sub>2</sub> ligands were used in the tissue culture bio-assay since they exhibited the greatest stability under the conditions of the bio-assay. Five out of ten Group A ligands tested inhibited TGF $\beta$ 1 receptor activity.  $IC_{50}$  values for the inhibitors were typically 25 fold above the  $K_d$  for TGF $\beta$ 1. The data are reproducible; the  $K_d$  for ligand lib3-13 was  $0.83 \pm 0.11$  nM ( $n=3$ ) and the  $IC_{50}$  for lib3-13 (SEQ ID NO:12) was  $25 \pm 14$  nM ( $n=4$ ). RNA concentrations in the bioassays are all estimates based on an assumed extinction coefficient and 100% purity of the ligand. The RNA concentrations may, therefore, be overestimated during the bio-assay which in turn would overestimate the  $IC_{50}$ .

Another five Group A ligands did not inhibit TGF $\beta$  receptor binding activity. One obvious difference between the non-bioactive ligands, lib2-6-4 (SEQ ID NO:20), lib5-48 (SEQ ID NO:19), and lib6-23 (SEQ ID NO:21), and the bioactive ligands is the substitution at nucleotide 72. Lib7-21 (SEQ ID NO:23) and lib7-43 (SEQ ID NO:24) were tested as 2'-F-UTP, 2'-NH<sub>2</sub>-CTP ligands for bio-activity. These ligands were not bio-active despite their high affinity to TGF $\beta$ . In conclusion, binding and bioactivity are separable functions of the TGF $\beta$  Group A ligands.

Group B ligands have different binding properties than Group A ligands (Table 4). Both the  $K_d$  ( $0.63 \pm 0.5$  nM,  $n=4$ ) and  $B_{max}$  ( $0.14 \pm 0.04$ ,  $n=4$ ) are lower for Group B ligands. One Group B inhibitor, lib4-12 (SEQ ID NO:28), actually appears to stimulate TGF $\beta$ 1 activity in the tissue culture bio-assay at low concentrations. The basis of this mixed agonist/antagonist behavior has not been

determined. The best inhibitor in this group, lib3-42 (SEQ ID NO:30) has an IC<sub>50</sub> of 22 nM and had no agonist behavior over the concentration ranges tested.

Group C ligands were tested as 2'-F derivatives and were not bio-active. Neither was the 2'-F orphan lib1-20-5 (SEQ ID NO:40). The 2'-NH<sub>2</sub>, 40N orphan, lib3-45 is an example of another ligand with high affinity for TGF $\beta$ 1 and no ability to inhibit TGF $\beta$ 1 receptor binding.

Group D ligands were tested in the bio-assay as 2'-NH<sub>2</sub>-UTP, 2'-F-CTP derivatives. Both lib2-6-1 (SEQ ID NO:35) and the truncated version lib2-6-1-81 (SEQ ID NO:36) can inhibit TGF $\beta$ 1 receptor binding; however, a single mutation from a C to a G at position 53 decreases bio-activity in clone lib8-23. Similarly a 2 base pair deletion in clone lib6-30 (SEQ ID NO:34) at positions corresponding to nucleotides 67 and 68 in lib2-6-1 (SEQ ID NO:35) increases binding by 10 fold but eliminates bio-activity.

Lib2-6-1 (SEQ ID NO:35) was shown to be fully effective only against TGF $\beta$ 1 and not TGF $\beta$ 2 and TGF $\beta$ 3. Lib2-6-1 (SEQ ID NO:35) was biologically active in the presence of 10% horse serum in the cell culture medium in addition to the 0.1% BSA. Thus the ligand demonstrates specificity towards TGF $\beta$ 1 which is not interfered with by the presence of the horse serum in this assay. The biggest indication that the inhibition of TGF $\beta$ 1 receptor binding is a specific phenomenon is the fact that not all TGF $\beta$ 1 ligands block receptor binding, but the ones that do, do so reproducibly. There are no examples of ligands that do not bind to TGF $\beta$ 1 blocking TGF $\beta$ 1 receptor binding activity.

In summary, RNA ligands that can block TGF $\beta$ 1 receptor binding are a subset of ligands. Binding is necessary but not sufficient for bio-activity. Roughly 50% of the high affinity ligands tested were inhibitors. Of the inhibitors, 30% were good inhibitors (IC<sub>50</sub> < 25 nM).

#### EXAMPLE 4. BOUNDARY ANALYSIS

Truncation experiments were done on a number of TGF $\beta$ 1 ligands to determine the nucleotides essential for binding. Group A ligands, lib3-13 (SEQ

ID NO:12) and lib8-9 (SEQ ID NO:16), were truncated with consistent results. The fragment lib3-13-79 binds to TGF $\beta$ 1, thus none of the nucleotides 3' to nucleotide 79 in lib3-13 are essential for binding. Similarly when all nucleotides 5' to nucleotide 38 are deleted the remaining fragment, lib3-13-(38-123) can still bind to TGF $\beta$ 1. The 5' boundary is in agreement with the sequence lib6-23 (SEQ ID NO:21), which has a deletion corresponding to nucleotides 19-36 of lib3-13 (SEQ ID NO:25), and still binds to TGF $\beta$ 1. Thus, all high affinity binding determinants for Group A clones may lie wholly within the random region and may correspond to a 42 nucleotide fragment, lib3-13-(38-79). Many Group A ligands contain deletions or substitutions within the predicted essential binding domain, in the region corresponding to lib3-13-(72-81). The deletion and substitution in lib4-32 have no effect on its 3' boundary which corresponds to lib3-13 nucleotide 80. Thus, the 3' boundary is probably correct and the alterations in nucleotide sequence 72-81 are ones that do not significantly alter the nucleic acid structure required for binding. Mutations in this region, most notably nucleotide 72 may, however, modify the ability of the ligand to block TGF $\beta$ 1 receptor binding as noted earlier.

Boundary analysis of the 3' end of Group B ligand, lib4-12 (SEQ ID NO:28), predicts that nothing beyond nucleotide 72 is required for TGF $\beta$ 1 binding. When the 5' boundary of lib4-12 was determined, all but the first three nucleotides were required for binding, indicating that the 5' constant region is an essential part of the ligand at least when the boundary of the full length ligand was determined. Assuming that ligand lib3-44 (SEQ ID NO:29) has a similar binding determinant as lib4-12 (SEQ ID NO:28), we can also conclude that nucleotides 37-46 of lib4-12 are not required for binding since these are deleted in lib3-44 and lib3-42 (SEQ ID NO:30).

The 3' constant region is not necessary for binding in Group C and D ligands. Both ligand types bind without the 3' nucleotides in the constant region. Lib1-20-3-82, an 82 nucleotide truncated version of lib1-20-3 (SEQ ID NO:32), binds as well as the full length lib1-20-3. Likewise binding and bioactivity of lib2-6-1 is unaffected by the 3' truncation found in lib2-6-1-81 (SEQ ID NO:36).

**EXAMPLE 5. EXPERIMENTAL PROCEDURES**

In the preferred embodiment, a second set of SELEX experiments was performed in search of RNA and DNA ligands with specific high affinity for TGF $\beta$ 1 from degenerate libraries containing 40 random positions (40N). This Example provides the general procedures followed and incorporated in Example 6.

**A. Materials.**

M-MLV superscript reverse transcriptase was purchased from Gibco BRL (Gaithersburg, MD). T7 RNA polymerase was purified according to standard procedures at NeXstar Pharmaceuticals, Inc. (Boulder, CO). Taq DNA polymerase (AmpliTaq) was from Perkin Elmer/Cetus (Richmond, CA). T4 polynucleotide kinase, DNA polymerase (Klenow fragment), and alkaline phosphatase were purchased from New England Biolabs, Inc. (Beverly, MA). The 2'-amino substituted nucleotide triphosphates amino-UTP and amino-CTP were synthesized according to standard procedures at NeXstar Pharmaceuticals, Inc. (Boulder, CO). Other reagents used in this work were of the highest quality obtainable.

**B. Nucleic Acids.**

RNAs were synthesized by *in vitro* transcription using double-stranded DNA oligonucleotides and T7 RNA polymerase. DNA oligonucleotides (Table 5) were purchased from Operon, Inc. (Alameda, CA) and purified by 6% preparative polyacrylamide gel electrophoresis. PCR amplification was performed in 50 mM KCl, 10 mM Tris-HCl (pH 8.6), 2.5 mM MgCl<sub>2</sub>, 170 mg/mL BSA, and dNTPs (present at 1 mM each). Taq DNA polymerase was used at 100 units per 0.1 mL reaction, and the 5'- and 3'-primers were present at 1 mM. Transcription was performed in 40 mM NaCl, 10 mM dithiothreitol, 50 mM Tris-acetate (pH 8.0), 8 mM magnesium acetate, 2 mM spermidine, and 2 mM NTP. T7 RNA polymerase was present at 1 unit/mL. The reaction was incubated at 28 degrees for 16 hours and then treated with 20 units of DNase I for an additional 10 min at 37 degrees. The reaction was stopped by the addition of one half volume of loading buffer

(93% formamide, 10 mM EDTA, pH 8.0) and heated to 95 degrees for 3 min prior to electrophoresis on a 6 % polyacrylamide/8 M urea denaturing gel. The RNA transcript was visualized by UV shadowing and was excised from the gel and eluted into TE buffer (10 mM Tris-acetate pH 8.0, 2 mM EDTA). The RNA transcript was ethanol precipitated, dried under vacuum, and redissolved in distilled H<sub>2</sub>O. The concentration of RNA as well as single-stranded DNA was quantified by measuring the A<sub>260</sub> and assuming that 1 A<sub>260</sub> unit equaled 40 mg/mL and 33 mg/mL, respectively.

10

### C. Evolution of High-Affinity Ligands.

SELEX ligands that bind to TGF $\beta$ 1 were derived essentially as described in U.S. Patent No. 5,270,163 (see also Tuerk and Gold (1990) Science 249:505-510) using the oligonucleotides illustrated in Table 5 (SEQ ID NOS:43-54). The DNA templates contained a 40-nucleotide (40N) variable sequence generated by mixed-nucleotide DNA synthesis, as well as 5'- and 3'-fixed sequences, necessary for PCR amplification of the template. The 5'-fixed sequence of oligonucleotides 40N7 (SEQ ID NO:43) and 40N8 (SEQ ID NO:49) also contained a T7 RNA polymerase promoter. RNA for the first round of RNA SELEX was transcribed from double-stranded DNA templates generated by primer extension on single-stranded DNA templates 40N7 and 40N8 with the Klenow fragment of DNA polymerase I. RNA SELEX consisted of up to 15 rounds of RNA synthesis, binding to target, partitioning of bound and unbound RNA by nitrocellulose filtration, cDNA synthesis, and PCR amplification to regenerate the double-stranded DNA template. Binding to the target by the RNA pool was performed in binding buffer A (120 mM NaCl, 2.5 mM KCl, 0.12 mM MgSO<sub>4</sub>, 40 mM HEPES, 20 mM NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> pH 7.4, 0.01% HSA) at 37 degrees for at least 10 min prior to filtration. In contrast, the first round of single-stranded DNA SELEX was performed by using the synthetically synthesized oligonucleotides 40D7 and 40D8 directly. SELEX consisted of 25 rounds of binding to target, partitioning of bound and unbound single-stranded DNA by nitrocellulose filtration, PCR amplification to generate a double-stranded

DNA population, and preparative polyacrylamide gel electrophoresis to purify single-stranded DNA for the next round of SELEX. Binding of the target to the single-stranded DNA pool was performed in binding buffer B (150 mM NaCl, 10 mM Tris-acetate pH 7.5, 0.001% BSA) at 37 degrees for at least 15 min prior to filtration. Radiolabeling of RNA as well as DNA repertoires was performed by incubation of 5 picomoles nucleic acid, 2 units of T4 polynucleotide kinase, and 6 mL [ $\gamma^{32}$ P] ATP (800 Ci/mmol) in a volume of 10 mL at 37 degrees for 30 min. The concentration of nucleic acid at each round of the SELEX experiment varied between 1500 nM and 1 nM while the concentration of the target TGF- $\beta$ 1 varied between 150 nM and 0.03 nM.

#### D. Cloning and Sequencing of Ligands.

Cloning of the nucleic acid repertoire was performed as described by Tuerk and Gold (1990) *Science* 249:505-510 using double-stranded DNA that was generated from the RNA repertoire by PCR amplification. PCR-amplified DNA was digested with the restriction enzymes SphI and HindIII and ligated into compatible sites within pGEM. Ligated plasmids were transformed into *E. coli* and plated onto LB agar containing 5-bromo-4-chloro-3-indolyl  $\beta$ -D-galactoside, isopropyl thiogalactoside, and 100 mg/mL ampicillin. Colonies not expressing  $\beta$ -galactosidase were analyzed. Sequencing of DNA was performed as described by Tuerk and Gold (1990) using the dideoxynucleotide procedure of Sanger *et al.* (1977) *Proc. Natl. Acad. Sci. USA* 74:5463-5467. Plasmids were isolated from *E. coli* by the alkaline lysis miniprep procedure (Manitatis *et al.* (1982) in Molecular Cloning: A laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). DNA was incubated in 50 mM Tris-HCl (pH 8.3), 60 mM NaCl, 6 mM magnesium acetate, and 1 mM DTT with 0.4 mM dNTP and 0.2 mM dideoxy-NTP for 20 min at 48 degrees. DNA polymerase was present at 4 units per reaction. The reactions were stopped by the addition of 10 mL of loading buffer and heated to 95 degrees for 3 min prior to gel electrophoresis on a 6% polyacrylamide/8 M urea denaturing gel. G-track sequencing was performed as described and provided a convenient method to quickly screen the cloned library for ligands of different sequence. Briefly, the G-track sequencing reaction

contained 50 mM Tris-HCl (pH 8.3), 60 mM NaCl, 6 mM magnesium acetate, and 1 mM DTT with 0.4 mM dNTP, 0.2 mM dideoxy-GTP, and 4 units of DNA polymerase. The reaction was performed at 48 degrees for 20 min and was stopped by the addition of 10 uL of loading buffer and heated to 95 degrees for 3 min prior to gel electrophoresis on a 6% polyacrylamide/8 M urea denaturing gel.

**EXAMPLE 6. BINDING ANALYSIS, BIOASSAY RESULTS, AND SEQUENCES OF A ssDNA LIBRARY.**

Binding analysis of the 40D7 DNA library for TGF-B1 is shown in Figure 10. Binding data obtained from round 19 (triangles) and round 0 (circles) are shown. The experiment was performed by incubating nucleic acid (less than 1 nM) and the indicated concentration of TGF-b1 in Binding Buffer (150 mM NaCl, 10 mM Tris-acetate pH 8.2, 0.001% BSA) for 15 minutes at 37 degrees in a volume of 0.1 mL. Samples were filtered through nitrocellulose and were 15 immediately followed by 3 mL of TE Buffer (10 mM Tris-acetate pH 8.0, 0.1 mM EDTA). The percentage of radiolabel bound was calculated from the amount of radiolabel retained on the nitrocellulose filter and the total radiolabel added to the binding reaction. The results show that the apparent *Kd* of the 40D7 library is 1 nM, whereas the starting pool has an apparent *Kd* of 30 nM. Thus, the 40D7 20 library shows an increase of about three fold in binding.

A PAI-luciferase assay to detect TGF-b1 activity in the presence of the nucleic acid libraries generated in Example 5 was performed as described in Abe *et al.* (1994) *Analytical Biochem.* 216:276-284. Mink lung epithelial cells containing the PAI-luciferase reporter gene were incubated with TGF-b1 (10 pM) 25 and oligonucleotides from the DNA libraries or anti -TGF-B antibody (60  $\mu$ g/mL). The mink lung epithelial cells were incubated for 18 hours and oligonucleotides were pre-incubated with TGF-b1 before the assay and readded after 8 hours. Addition of oligonucleotides alone (100 nM) to the cell culture did not affect the 30 assay (data not shown). The identity of the oligonucleotide libraries as well as their effect on luciferase activity is indicated in Figure 2. The ssDNA library 40N7 completely inhibited the activity of TGF-B1, while the control (an equal

concentration of randomized nucleic acid) showed a small stimulation of TGF-B1 activity.

Based on the results of the binding analysis and PAI-luciferase assay, DNA ligands from the 40N7 library were sequenced as described in Example 5.

5 The sequences are shown in Table 6 (SEQ ID NOS:55-89). As the DNA 40N7 library showed inhibition in the PAI-luciferase bioassay, it is reasonable to suggest that the individual clones from the library are TGF $\beta$ 1 binders.

#### **EXAMPLE 7. EXPERIMENTAL PROCEDURES**

10 This Example provides the general procedures followed and incorporated in Examples 8-15 for the evolution of nucleic acid ligands to PDGF.

##### **A. Materials.**

Recombinant human PDGF-AA ( $M_r=29,000$ ), PDGF-AB ( $M_r=27,000$ ) and PDGF-BB ( $M_r=25,000$ ) were purchased from R&D Systems (Minneapolis, 15 MN) in lyophilized form, free from carrier protein. All three isoforms were produced in *E. coli* from synthetic genes based on the sequences for the long form of the mature human PDGF A-chain (Betsholtz *et al.*, (1986) *Nature* 320: 695-699) and the naturally occurring mature form of human PDGF B-chain (Johnsson *et al.*, (1984) *EMBO J.* 3: 921-928). Randomized DNA libraries, PCR primers and DNA ligands and 5'-iodo-2'-deoxyuridine-substituted DNA ligands were synthesized by NeXstar Pharmaceuticals, Inc. (Boulder, CO) or by Operon Technologies (Alameda, CA) using the standard solid phase phosphoramidite method (Sinha *et al.*, (1984) *Nucleic Acids Res.* 12: 4539-4557).

##### **B. Single Stranded DNA (ssDNA) Selex**

25 Essential features of the SELEX procedure have been described in detail in the SELEX Patent Applications (see also, Tuerk and Gold, *Science*, 249: 505 (1990); Jellinek *et al.*, *Biochemistry*, 33: 10450 (1994); Jellinek *et al.*, *Proc. Natl. Acad. Sci.*, 90: 11227 (1993)), which are incorporated by reference herein. The initial ssDNA library containing a contiguous randomized region of forty 30 nucleotides, flanked by primer annealing regions (Table 7; SEQ ID NO:90) of invariant sequence, was synthesized by the solid phase phosphoramidite method

using equal molar mixture of the four phosphoramidites to generate the randomized positions. The ssDNA library was purified by electrophoresis on an 8% polyacrylamide/7 M urea gel. The band that corresponds to the full-length DNA was visualized under UV light, excised from the gel, eluted by the crush and soak method, ethanol precipitated and pelleted by centrifugation. The pellet was dried under vacuum and resuspended in phosphate buffered saline supplemented with 1 mM MgCl<sub>2</sub> (PBSM = 10.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl and 2.7 mM KCl, 1 mM MgCl<sub>2</sub>, pH 7.4) buffer. Prior to incubation with the protein, the ssDNA was heated at 90°C for 2 minutes in PBSM and cooled on ice.

The first selection was initiated by incubating approximately 500 pmol ( $3 \times 10^{14}$  molecules) of 5' <sup>32</sup>P end-labeled random ssDNA with PDGF-AB in binding buffer (PBSM containing 0.01% human serum albumin (HSA)). The mixture was incubated at 4°C overnight, followed by a brief (15 min) incubation at 37°C. The DNA bound to PDGF-AB was separated from unbound DNA by electrophoresis on an 8% polyacrylamide gel (1:30 bis-acrylamide:acrylamide) at 4°C and at 5 V/cm with 89 mM Tris-borate (pH 8.3) containing 2 mM EDTA as the running buffer. The band that corresponds to the PDGF-ssDNA complex, which runs with about half the electrophoretic mobility of the free ssDNA, was visualized by autoradiography, excised from the gel and eluted by the crush and soak method.

In subsequent affinity selections, the ssDNA was incubated with PDGF-AB for 15 minutes at 37°C in binding buffer and the PDGF-bound ssDNA was separated from the unbound DNA by nitrocellulose filtration, as previously described (Green,*et al.*, (1995) *Chemistry and Biology* 2, 683-695). All affinity-selected ssDNA pools were amplified by PCR in which the DNA was subjected to 12-20 rounds of thermal cycling (30 s at 93°C, 10 s at 52°C, 60 s at 72°C) in 10 mM Tris-Cl (pH 8.4) containing 50 mM KCl, 7.5 mM MgCl<sub>2</sub>, 0.05 mg/ml bovine serum albumin, 1 mM deoxynucleoside triphosphates, 5 μM primers (Table 7) and 0.1 units/μl Taq polymerase. The 5' PCR primer was 5' end-labeled with polynucleotide kinase and [ $\gamma$ -<sup>32</sup>P]ATP and the 3' PCR primer was biotinylated at the 5' end using biotin phosphoramidite (Glen Research, Sterling, VA). Following PCR amplification, streptavidin (Pierce, Rockford, IL) was added to the

unpurified PCR reaction mixture at a 10-fold molar excess over the biotinylated primer and incubated for 15 min at room temperature. The dsDNA was denatured by adding an equal volume of stop solution (90% formamide, 1% sodium dodecyl sulfate, 0.025% bromophenol blue and xylene cyanol) and incubating for 20 min at room temperature. The radiolabeled strand was separated from the streptavidin-bound biotinylated strand by electrophoresis on 12% polyacrylamide/7M urea gels. The faster migrating radiolabeled (non-biotinylated) ssDNA strand was cut out of the gel and recovered as described above. The amount of ssDNA was estimated from the absorbance at 260 nm using the extinction coefficient of 33  $\mu\text{g}/\text{ml}/\text{absorbance unit}$  (Sambrook *et al.*, 1989) *Molecular Cloning: A Laboratory Manual*, 2 Ed. 3 vols., Cold Spring Harbor Laboratory Press, Cold Spring Harbor).

**C. Cloning and Sequencing.**

The amplified affinity-enriched pool from SELEX round 12 was purified on a 12% polyacrylamide gel and cloned between *Hind*III and *Pst*I sites in JM109 strain of *E. coli* (Sambrook, *et al.*, (1989) *Molecular Cloning: A Laboratory Manual*, 2 Ed. 3 vols., Cold Spring Harbor Laboratory Press, Cold Spring Harbor). Individual clones were used to prepare plasmids by alkaline lysis. Plasmids were sequenced at the insert region using the forward sequencing primer and Sequenase 2.0 (Amersham, Arlington Heights, IL) according to the manufacturer's protocol.

**D. Determination of the apparent equilibrium dissociation constants and the dissociation rate constants.**

The binding of ssDNA ligands at low concentrations to varying concentrations of PDGF was determined by the nitrocellulose filter binding method as described (Green *et al.*, (1995) *Chemistry and Biology* 2: 683-695). The concentrations of PDGF stock solutions (in PBS) were determined from the absorbance readings at 280 nm using the following  $\epsilon_{280}$  values calculated from the amino acid sequences (Gill, S. C., and von Hippel, P. H. (1989) *Anal. Biochem.* 182: 319-326): 19,500  $\text{M}^{-1}\text{cm}^{-1}$  for PDGF-AA, 15,700  $\text{M}^{-1}\text{cm}^{-1}$  for PDGF-AB and 11,800  $\text{M}^{-1}\text{cm}^{-1}$  for PDGF-BB. ssDNA for all binding experiments were purified by electrophoresis on 8% (>80 nucleotides) or 12% (<40 nucleotides)

polyacrylamide/7 M urea gels. All ssDNA ligands were heated at 90°C in binding buffer at high dilution ( $\approx 1$  nM) for 2 min and cooled on ice prior to further dilution into the protein solution. The binding mixtures were typically incubated for 15 min at 37°C before partitioning on nitrocellulose filters.

5       The binding of DNA ligands (L) to PDGF-AA (P) is adequately described with the bimolecular binding model for which the fraction of bound DNA at equilibrium (q) is given by eq. 1,

$$q = \frac{f}{2} \frac{([P]_t + [L]_t + K_d - \sqrt{([P]_t + [L]_t + K_d)^2 - 4[P]_t [L]_t})^{1/2}}{[P]_t} \quad (1)$$

10

where  $[P]_t$  and  $[L]_t$  are total protein and total DNA concentrations,  $K_d$  is the equilibrium dissociation constant and f is the efficiency of retention of protein-DNA complexes on nitrocellulose filters (Irvine *et al.*, (1991) *J. Mol. Biol.* **222**: 739-761; Jellinek *et al.*, (1993) *Proc. Nat'l. Acad. Sci. USA* **90**: 11227-11231).

15

The binding of DNA ligands to PDGF-AB and PDGF-BB is biphasic and can be described by a model in which the DNA ligand is composed of two non-interconverting components ( $L_1$  and  $L_2$ ) that bind to the protein with different affinities, described by corresponding dissociation constants,  $K_{d1}$  and  $K_{d2}$  (Jellinek *et al.*, 1993) *Proc. Nat'l. Acad. Sci. USA* **90**: 11227-11231). In this case, the explicit solution for the fraction of bound DNA (q) is given by eq. 2,

$$q = f \left( \frac{\chi_1 K_{d1}}{1 + K_{d1} [P]} + \frac{\chi_2 K_{d2}}{1 + K_{d2} [P]} \right) [P] \quad (2)$$

with

$$[P] = \frac{[P]_t}{1 + \frac{\chi_1 K_{d1} [L]_1}{1 + K_{d1} [P]} + \frac{\chi_2 K_{d2} [L]_2}{1 + K_{d2} [P]}}$$

where  $\chi_1$  and  $\chi_2 (=1-\chi_1)$  are the mole fractions of  $L_1$  and  $L_2$ . The  $K_d$  values for the binding of DNA ligands to PDGF were calculated by fitting the data points to eq. 1 (for PDGF-AA) or eq. 2 (for PDGF-AB and PDGF-BB) using the non-linear least squares method.

5       The dissociation rate constants ( $k_{off}$ ) were determined by measuring the amount of  $^{32}P$  5'-end labeled minimal ligands (0.17 nM) bound to PDGF-AB (1 nM) as a function of time following the addition of 500-fold excess of unlabeled ligands, using nitrocellulose filter binding as the partitioning method. The  $k_{off}$  values were determined by fitting the data points to the first-order rate equation  
10      (eq. 3)

$$(q-q_\infty)/(q_0-q_\infty) = \exp(-k_{off}t) \quad (3)$$

15      where  $q$ ,  $q_0$  and  $q_\infty$  represent the fractions of DNA bound to PDGF-AB at any time ( $t$ ),  $t=0$  and  $t=\infty$ , respectively.

#### E. Minimal ligand determinations.

To generate a population of 5' end-labeled DNA ligands serially truncated from the 3' end, a primer complementary to the 3' invariant sequence region of a DNA ligand template (truncated primer 5N2, Table 7; SEQ ID NO:92) was radiolabeled at the 5' end with [ $\gamma$ - $^{32}P$ ]-ATP and T4 polynucleotide kinase, annealed to the template and extended with Sequenase (Amersham, Arlington Heights, IL) and a mixture of all four dNTPs and ddNTPs. Following incubation in binding buffer for 15 min at 37°C, the fragments from this population that retain high affinity binding to PDGF-AB were separated from those with weaker affinity by nitrocellulose filter partitioning. Electrophoretic resolution of the fragments on 8% polyacrylamide/7 M urea gels, before and after affinity selection, allows determination of the 3' boundary. To generate a population of 3' end-labeled DNA ligands serially truncated from the 5' end, the DNA ligands were radiolabeled at the 3' end with [ $\alpha$ - $^{32}P$ ]-cordycepin-5'-triphosphate (New England Nuclear, Boston, MA) and T4 RNA ligase (Promega, Madison, WI), phosphorylated at the 5' end with ATP and T4 polynucleotide kinase, and partially

5 digested with lambda exonuclease (Gibco BRL, Gaithersburg, MD). Partial digestion of 10 pmols of 3'-labeled ligand was done in 100  $\mu$ L volume with 7 mM glycine-KOH (pH 9.4), 2.5mM MgCl<sub>2</sub>, 1  $\mu$ g/ml BSA, 15  $\mu$ g tRNA, and 4 units of lambda exonuclease for 15 min at 37°. The 5' boundary was determined in an analogous manner to that described for the 3' boundary.

**F. Melting temperature ( $T_m$ ) measurements.**

10 Melting profiles for the minimal DNA ligands were obtained on a Cary Model 1E spectrophotometer. Oligonucleotides (320-400 nM) were heated to 95°C in PBS, PBSM or PBS with 1 mM EDTA and cooled to room temperature prior to the melting profile determination. Melting profiles were generated by heating the samples at the rate of 1 °C/min from 15-95°C and recording the absorbance every 0.1 °C. The first derivative of the data points was calculated using the plotting program KaleidaGraph (Synergy Software, Reading, PA). The first derivative values were smoothed using a 55 point smoothing function by averaging each point with 27 data points on each side. The peak of the smoothed first derivative curves was used to estimate the  $T_m$  values.

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**G. Crosslinking of 5-iodo-2'-deoxyuridine-substituted DNA ligands to PDGF-AB.**

20 DNA ligands containing single or multiple substitutions of 5'-ido-2'deoxyuridine for thymidine were synthesized using the solid phase phosphoramidite method. To test for the ability to crosslink, trace amounts of 5'<sup>32</sup>P end-labeled ligands were incubated with PDGF-AB (100 nM) in binding buffer at 37° for 15 min prior to irradiation. The binding mixture was transferred to a 1 cm path length cuvette thermostated at 37° and irradiated at 308 nm for 25-400 s at 20 Hz using a XeCl charged Lumonics Model EX748 excimer laser. The cuvette was positioned 24 cm beyond the focal point of a convergent lens, with the energy at the focal point measuring 175 mjoules/pulse. Following 25 irradiation, aliquots were mixed with an equal volume of formamide loading buffer containing 0.1% SDS and incubated at 95° for 5 min prior to resolution of the crosslinked PDGF/ligand complex from the free ligand on 8% polyacrylamide/7 M urea gels.

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To identify the protein site of crosslinking for ligand 20t-I4, binding and irradiation were done on a larger scale. PDGF-AB and 5' <sup>32</sup>P end-labeled ligand, each at 1  $\mu$ M in PBSM, were incubated and irradiated (300 s) as described above in two 1 ml reaction vessels. The reaction mixtures were combined, ethanol precipitated and resuspended in 0.3 ml of Tris-HCl buffer (100mM, pH 8.5). The PDGF-AB/ligand crosslinked complex was digested with 0.17  $\mu$ g/ $\mu$ l of modified trypsin (Boehringer Mannheim) for 20 hours at 37°. The digest mixture was extracted with phenol/chloroform, chloroform and then ethanol precipitated. The pellet was resuspended in water and an equal volume of formamide loading buffer with 5% (v/v)  $\beta$ -mercaptoethanol (no SDS), incubated at 95° for 5 min, and resolved on a 40 cm 8% polyacrylamide/7 M urea gel. The crosslinked tryptic-peptide/ligand that migrated as two closely spaced bands about 1.5 cm above the free ligand band was excised from the gel and eluted by the crush and soak method and ethanol precipitated. The dried crosslinked peptide (about 160 pmoles based on the specific activity) was sequenced by Edman degradation (Midwest Analytical, Inc., St. Louis, MO).

#### H. Receptor Binding Assay.

The binding of <sup>125</sup>I-PDGF-AA and <sup>125</sup>I-PDGF-BB to porcine aortic endothelial (PAE) cells transfected with PDGF  $\alpha$ - or  $\beta$ -receptors were performed as described (Heldin *et al.*, (1988) EMBO J. 7, 1387-1394). Different concentrations of DNA ligands were added to the cell culture (1.5 cm<sup>2</sup>) in 0.2 ml of phosphate buffered saline supplemented with 1 mg bovine serum albumin per ml together with <sup>125</sup>I-PDGF-AA (2 ng, 100,000 cpm) or <sup>125</sup>I-PDGF-BB (2 ng, 100,000 cpm). After incubation at 4°C for 90 min, the cell cultures were washed and cell associated radioactivity determined in a  $\gamma$ -counter (Heldin *et al.*, (1988) EMBO J. 7, 1387-1394).

#### L. [<sup>3</sup>H]thymidine Incorporation Assay.

The incorporation of [<sup>3</sup>H]thymidine into PAE cells expressing PDGF  $\beta$ -receptor in response to 20 ng/ml of PDGF-BB or 10% fetal calf serum and in the presence of different concentrations of DNA ligands was performed as described

(Mori *et al.*, (1991) *J. Biol. Chem.* **266**, 21158-21164). After incubation for 24 h at 37°C, <sup>3</sup>H-radioactivity incorporated into DNA was determined using a β-counter.

5        **EXAMPLE 8. ssDNA LIGANDS OF PDGF**

High affinity DNA ligands to PDGF AB were identified by the SELEX process from a library of =  $3 \times 10^{14}$  molecules (500 pmol) of single stranded DNA randomized at forty contiguous positions (Table 7; SEQ ID NO:90). The 10 PDGF-bound DNA was separated from unbound DNA by polyacrylamide gel electrophoresis in the first round and by nitrocellulose filter binding in the subsequent rounds. After 12 rounds of SELEX, the affinity-enriched pool bound to PDGF-AB with an apparent dissociation constant ( $K_d$ ) of ≈ 50 pM (data not shown). This represented an improvement in affinity of ≈ 700-fold compared to 15 the initial randomized DNA library. This affinity-enriched pool was used to generate a cloning library from which 39 isolates were sequenced. Thirty-two of these ligands were found to have unique sequences (Table 8; SEQ ID NOS:93-124). Ligands that were subjected to the minimal sequence determination are marked with an asterisk (\*) next to the clone number. The clone numbers that 20 were found to retain high affinity binding as minimal ligands are italicized. All ligands shown in Table 8 were screened for their ability to bind to PDGF AB using the nitrocellulose filter binding method. To identify the best ligands from this group, we determined their relative affinities for PDGF-AB by measuring the 25 fraction of 5' <sup>32</sup>P end-labeled ligands bound to PDGF-AB over a range of protein concentrations. For the ligands that bound to PDGF-AB with high affinity, the affinity toward PDGF-BB and PDGF-AA was also examined: in all cases, the affinity of ligands for PDGF-AB and PDGF-BB was comparable while the affinity for PDGF-AA was considerably lower (data not shown).

Twenty-one of the thirty-two unique ligands can be grouped into a 30 sequence family shown in Table 9. The sequences of the initially randomized region (uppercase letters) are aligned according to the consensus three-way helix

junction motif. Nucleotides in the sequence-invariant region (lowercase letters) are only shown where they participate in the predicted secondary structure.

Several ligands were "disconnected" (equality symbol) in order to show their relatedness to the consensus motif through circular permutation. The nucleotides predicted to participate in base pairing are indicated with underline inverted arrows, with the arrow heads pointing toward the helix junction. The sequences are divided into two groups, A and B, based on the first single stranded nucleotide (from the 5' end) at the helix junction (A or G, between helices II and III).

Mismatches in the helical regions are shown with dots under the corresponding letters (G-T and T-G base pairs were allowed). In places where single nucleotide bulges occur, the mismatched nucleotide is shown above the rest of the sequence between its neighbors.

This classification is based in part on sequence homology among these ligands, but in greater part on the basis of a shared secondary structure motif: a three-way helix junction with a three nucleotide loop at the branch point (Figure 3). These ligands were subdivided into two groups; for ligands in group A, the loop at the branch point has an invariant sequence AGC and in group B, that sequence is G(T/G)(C/T). The proposed consensus secondary structure motif is supported by base-pairing covariation at non-conserved nucleotides in the helices (Table 10). Since the three-way junctions are encoded in continuous DNA strands, two of the helices end in loops at the distal end from the junction. These loops are highly variable, both in length and in sequence. Furthermore, through circular permutation of the consensus motif, the loops occur in all three helices, although they are most frequent in helices II and III. Together these observations suggest that the regions distal from the helix junction are not important for high affinity binding to PDGF-AB. The highly conserved nucleotides are indeed found near the helix junction (Table 9, Figure 3).

#### EXAMPLE 9. BOUNDARY ANALYSIS

The minimal sequence necessary for high affinity binding was determined for the six best ligands to PDGF-AB. In general, the information about the 3' and

5' minimal sequence boundaries can be obtained by partially fragmenting the nucleic acid ligand and then selecting for the fragments that retain high affinity for the target. With RNA ligands, the fragments can be conveniently generated by mild alkaline hydrolysis (Tuerk *et al.*, (1990) *J. Mol. Biol.* **213**: 749-761; Jellinek *et al.*, (1994) *Biochemistry* **33**: 10450-10456; Jellinek *et al.*, (1995) *Biochemistry* **34**: 11363-11372; Green *et al.*, (1995) *J. Mol. Biol.* **247**: 60-68). Since DNA is more resistant to base, an alternative method of generating fragments is needed for DNA. To determine the 3' boundary, a population of ligand fragments serially truncated at the 3' end was generated by extending the 5' end-labeled primer annealed to the 3' invariant sequence of a DNA ligand using the dideoxy sequencing method. This population was affinity-selected by nitrocellulose filtration and the shortest fragments (truncated from the 3' end) that retain high affinity binding for PDGF-AB were identified by polyacrylamide gel electrophoresis. The 5' boundary was determined in an analogous manner except that a population of 3' end-labeled ligand fragments serially truncated at the 5' end was generated by limited digestion with lambda exonuclease. The minimal ligand is then defined as the sequence between the two boundaries. It is important to keep in mind that, while the information derived from these experiments is useful, the suggested boundaries are by no means absolute since the boundaries are examined one terminus at a time. The untruncated (radiolabeled) termini can augment, reduce or have no effect on binding (Jellinek *et al.*, (1994) *Biochemistry* **33**: 10450-10456).

Of the six minimal ligands for which the boundaries were determined experimentally, two (20t (SEQ ID NO:172) and 41t (SEQ ID NO:174); truncated versions of ligands 20 and 41) bound with affinities comparable (within a factor of 2) to their full-length analogs and four had considerably lower affinities. The two minimal ligands that retained high affinity binding to PDGF, 20t and 41t, contain the predicted three-way helix junction secondary structure motif (Figure 4). The sequence of the third minimal ligand that binds to PDGF-AB with high affinity, 36t (SEQ ID NO:173), was deduced from the knowledge of the consensus motif (Figure 4). In subsequent experiments, we found that the single-stranded region at

the 5' end of ligand 20t is not important for high affinity binding. Furthermore, the trinucleotide loops on helices II and III in ligand 36t (GCA and CCA) can be replaced with pentaethylene glycol spacers (*infra*). These experiments provide further support for the importance of the helix junction region in high affinity binding to PDGF-AB.

The binding of minimal ligands 20t, 36t, and 41t to varying concentrations of PDGF-AA, PDGF-AB and PDGF-BB is shown in Figures 5A, 5B and 5C. In agreement with the binding properties of their full length analogs, the minimal ligands bind to PDGF-AB and PDGF-BB with substantially higher affinity than to PDGF AA (Figures 5A, 5B, and 5C, Table 11). In fact, their affinity for PDGF-AA is comparable to that of random DNA (data not shown). The binding to PDGF-AA is adequately described with a monophasic binding equation while the binding to PDGF-AB and PDGF-BB is notably biphasic. In previous SELEX experiments, biphasic binding has been found to be a consequence of the existence of separable nucleic acid species that bind to their target protein with different affinities (Jellinek *et al.*, (1995) *Biochemistry* **34**: 11363-11372) and unpublished results). The identity of the high and the low affinity fractions is at present not known. Since these DNA ligands described here were synthesized chemically, it is possible that the fraction that binds to PDGF-AB and PDGF-BB with lower affinity represents chemically imperfect DNA. Alternatively, the high and the low affinity species may represent stable conformational isomers that bind to the PDGF B-chain with different affinities. In any event, the higher affinity binding component is the most populated ligand species in all cases (Figures 5B and 5C). For comparison, a 39-mer DNA ligand that binds to human thrombin with a  $K_d$  of 0.5 nM (ligand T39 (SEQ ID NO.:177):  
5'-CAGTCCGTGGTAGGGCAGGTTGGGTGACTTCGTGGAA[3'T], where [3'T] represents a 3'-3' linked thymidine nucleotide added to reduce 3'-exonuclease degradation) and has a predicted stem-loop structure, binds to PDGF-AB with a  $K_d$  of 0.23  $\mu$ M (data not shown).

**EXAMPLE 10. KINETIC STABILITY OF PDGF-NUCLEIC ACID LIGAND COMPLEXES**

In order to evaluate the kinetic stability of the PDGF-AB/DNA complexes, the dissociation rates were determined at 37°C for the complexes of minimal ligands 20t, 36t and 41t (SEQ ID NOS:172-174) with PDGF-AB by measuring the amount of radiolabeled ligands (0.17 nM) bound to PDGF-AB (1 nM) as a function of time following the addition of a large excess of unlabeled ligands (Figure 6). At these protein and DNA ligand concentrations, only the high affinity fraction of the DNA ligands binds to PDGF-AB. The following values for the dissociation rate constants were obtained by fitting the data points shown in Figure 6 to the first-order rate equation:  $4.5 \pm 0.2 \times 10^{-3} \text{ s}^{-1}$  ( $t_{1/2} = 2.6 \text{ min}$ ) for ligand 20t,  $3.0 \pm 0.2 \times 10^{-3} \text{ s}^{-1}$  ( $t_{1/2} = 3.8 \text{ min}$ ) for ligand 36t, and  $1.7 \pm 0.1 \times 10^{-3} \text{ s}^{-1}$  ( $t_{1/2} = 6.7 \text{ min}$ ) for ligand 41t. The association rates calculated for the dissociation constants and dissociation rate constants ( $k_{on} = k_{off}/K_d$ ) are  $3.1 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$  for 20t,  $3.1 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$  for 36t and  $1.2 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$  for 41t.

**EXAMPLE 11. THERMAL MELTING PROPERTIES**

In order to examine the ability of minimal ligands 20t, 36t and 41t to assume folded structures, their melting temperatures ( $T_m$ 's) were determined from the UV absorbance vs. temperature profiles in PBSM or PBSE buffers. At the oligonucleotide concentrations used in these experiments (320-440 nM), only the monomeric species were observed as single bands on non-denaturing polyacrylamide gels (data not shown). Ligands 20t and 41t underwent thermal melting that is well described by a two-state (folded and unfolded) model with linearly sloping baselines (Petersheim and Turner (1983) *Biochem.* **22**:256-263) with  $T_m$  values in PBSM buffer of  $43.8 \pm 0.4 \text{ }^\circ\text{C}$  and  $49.2 \pm 0.5 \text{ }^\circ\text{C}$ , respectively. In PBSE buffer, similar  $T_m$  values were obtained:  $44.8 \pm 0.5 \text{ }^\circ\text{C}$  for ligand 20t and  $48.0 \pm 0.5 \text{ }^\circ\text{C}$  for ligand 41t. Ligand 36t exhibited a more complex thermal melting profile in which two distinct transitions were observed. In this case, the data were well described by a three-state model in which the fully folded and the unfolded states are connected through a partially unfolded intermediate results. Using this model, we obtained two  $T_m$  values for ligand 36t:  $47.0 \pm 0.9 \text{ }^\circ\text{C}$  and

67.1 ± 3.8 °C in PBSM buffer and 44.2 ± 1.7 °C and 64.3 ± 4.1 °C in PBSE buffer.

5           **EXAMPLE 12. PHOTO-CROSSLINKING OF NUCLEIC ACID LIGANDS AND PDGF**

In order to determine the sites on the DNA ligands and PDGF that are in close contact, a series of photo-crosslinking experiments were performed with 5'-iodo-2'-deoxyuridine (IdU)-substituted DNA ligands 20t, 36t and 41t (SEQ ID NOS:172-174). Upon monochromatic excitation at 308 nm, 5-iodo- and 10 5-bromo-substituted pyrimidine nucleotides populate a reactive triplet state following intersystem crossing from the initial n to  $\pi^*$  transition. The excited triplet state species then reacts with electron rich amino acid residues (such as Trp, Tyr and His) that are in its close proximity to yield a covalent crosslink. This method has been used extensively in studies of nucleic acid-protein interactions 15 since it allows irradiation with >300 nm light which minimizes photodamage (Willis *et al.*, (1994) *Nucleic Acids Res.* **22**: 4947-4952; Stump, W. T., and Hall, K. B. (1995) *RNA* **1**: 55-63; Willis *et al.*, (1993) *Science* **262**: 1255-1257; Jensen *et al.*, (1995) *Proc. Natl. Acad. Sci., U. S. A.* **92**: 12220-12224). Analogs 20 of ligands 20t, 36t and 41t were synthesized in which all thymidine residues were replaced with IdU residues using the solid phase phosphoramidite method. The 20 affinity of these IdU-substituted ligands for PDGF-AB was somewhat enhanced compared to the unsubstituted ligands and based on the appearance of bands with slower electrophoretic mobility on 8% polyacrylamide/7 M urea gels, all three 5' end-labeled IdU-substituted ligands crosslinked to PDGF-AB upon irradiation at 25 308 nm (data not shown). The highest crosslinking efficiency was observed with IdU-substituted ligand 20t. To identify the specific IdU position(s) responsible for the observed crosslinking, seven singly or multiply IdU-substituted analogs of 20t were tested for their ability to photo-crosslink to PDGF-AB: ligands 20t-11 through 20t-17

30 (5'-TGGGAGGGCGCGT'T'CT'T'CGT'GGT'T'ACT'S'T'6T'6AGT'CCCG-3' (SEQ ID NOS:178-184) where the numbers indicate IdU substitutions at indicated

thymidine nucleotides for the seven ligands). Of these seven ligands, efficient crosslinking to PDGF-AB was observed only with ligand 20t-14. The photo-reactive IdU position corresponds to the 3' proximal thymidine in the loop at the helix junction (Figure 4).

To identify the crosslinked amino acid residue(s) on PDGF-AB, a mixture of 5' end-labeled 20t-14 and PDGF-AB was incubated for 15 min at 37°C followed by irradiation at 308 nm. The reaction mixture was then digested with modified trypsin and the crosslinked fragments resolved on an 8% polyacrylamide/7 M urea gel. Edman degradation of the peptide fragment recovered from the band that migrated closest to the free DNA band revealed the amino acid sequence KKPIXKK (SEQ ID NO:185), where X indicates a modified amino acid that could not be identified with the 20 derivatized amino acid standards. This peptide sequence, where X is phenylalanine, corresponds to amino acids 80-86 in the PDGF-B chain (Johnsson *et al.*, (1984) *EMBO J.* 3: 921-928) which in the crystal structure of PDGF-BB comprises a part of solvent-exposed loop III (Oefner *et al.*, (1992) *EMBO J.* 11: 3921-3926). In the PDGF A-chain, this peptide sequence does not occur (Betsholtz *et al.*, (1986) *Nature* 320, 695-699). Together, these data establish a point contact between a specific thymidine residue in ligand 20t and phenylalanine 84 of the PDGF B-chain.

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#### EXAMPLE 13. INHIBITION OF PDGF BY NUCLEIC ACID LIGANDS

In order to determine whether the DNA ligands to PDGF were able to inhibit the effects of PDGF isoforms on cultured cells, the effects on binding of <sup>125</sup>I-labeled PDGF isoforms to PDGF  $\alpha$ - and  $\beta$ -receptors stably expressed in porcine aortic endothelial (PAE) cells by transfection was determined. Ligands 20t, 36t and 41t (SEQ ID NOS:172-174) all efficiently inhibited the binding of <sup>125</sup>I-PDGF-BB to PDGF  $\alpha$ -receptors (Figure 7) or PDGF  $\beta$ -receptors (data not shown), with half maximal effects around 1 nM of DNA ligand. DNA ligand T39, directed against thrombin and included as a control, showed no effect. None of the ligands was able to inhibit the binding of <sup>125</sup>I-PDGF-AA to the PDGF  $\alpha$ -receptor (Figure 7), consistent with the observed specificity of ligands 20t, 36t

and 41t for PDGF-BB and PDGF-AB.

The ability of the DNA ligands to inhibit the mitogenic effects of PDGF-BB on PAE cells expressing PDGF  $\beta$ -receptors was investigated. As shown in Figure 8, the stimulatory effect of PDGF-BB on [ $^3$ H]thymidine incorporation was neutralized by ligands 20t, 36t and 41t. Ligand 36t exhibited half maximal inhibition at the concentration of 2.5 nM; ligands 41t was slightly more efficient and 20t slightly less efficient. The control ligand T39 had no effect. Moreover, none of the ligands inhibited the stimulatory effects of fetal calf serum on [ $^3$ H]thymidine incorporation in these cells, showing that the inhibitory effects are specific for PDGF.

**EXAMPLE 14. POST-SELEX PROCESS NUCLEOTIDE SUBSTITUTIONS**

The stability of nucleic acids to nucleases is an important consideration in efforts to develop nucleic acid-based therapeutics. Experiments have shown that many, and in some cases most of the nucleotides in SELEX-derived ligands can be substituted with modified nucleotides that resist nuclease digestion, without compromising high affinity binding (Green *et al.*, (1995) Chemistry and Biology 2: 683-695; Green *et al.*, (1995) J. Mol. Biol. 247, 60-68). Experiments of this type with the DNA ligands reported here suggest that substitutions with modified nucleotides are tolerated at many positions (Figure 9; SEQ ID NOS:175-176). Specifically, we have examined the substitution of 2'-O-methyl-2'-deoxy- and 2'-fluoro-2'-deoxyribonucleotides for 2'-deoxyribonucleotides in ligand 36t, by examining the PDGF-AB binding properties of singly or multiply substituted ligand 36t. The substitution pattern indicated in Figure 9 is compatible with high affinity binding to PDGF-AB. Furthermore, this ligand tolerates the substitution of pentaethylene glycol spacers (Glen Research, Sterling, VA) for the trinucleotide loops at the ends of helices II and III (Figure 9). These DNA ligands therefore represent lead compounds for a novel class of high affinity, specific antagonists of PDGF-AB and PDGF-BB.

**EXAMPLE 15. EXPERIMENTAL PROCEDURE FOR EVOLVING  
2'-FLUORO-2'-DEOXYPYRIMIDINE RNA LIGANDS TO PDGF AND  
RNA SEQUENCES OBTAINED.**

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**A. 2'-FLUORO-2'-DEOXYPYRIMIDINE RNA SELEX**

SELEX with 2'-fluoro-2'-deoxypyrimidine RNA targeting PDGF AB was done essentially as described previously (*vide supra*, and Jellinek *et al.*, (1993, 1994) *supra*) using the primer template set as shown in Table 12 (SEQ ID NOS:125-127). Briefly, the 2'-fluoro-2'-deoxypyrimidine RNA for affinity selections was prepared by *in vitro* transcription from synthetic DNA templates using T7 RNA polymerase (Milligan *et al.*, *Nucl. Acids Res.*, **15**: 8783 (1987)). The conditions for *in vitro* transcription described in detail previously (Jellinek *et al.*, (1994) *supra*) were used, except that higher concentration (3 mM) of the 2'-fluoro-2'-deoxypyrimidine nucleoside triphosphates (2'-F-UTP and 2'-F-CTP) was used compared to ATP and GTP (1 mM). Affinity selections were done by incubating PDGF AB with 2'-fluoro-2'-deoxypyrimidine RNA for at least 15 min at 37 °C in PBS containing 0.01% human serum albumin. Partitioning of free RNA from protein-bound RNA was done by nitrocellulose filtration as described (Jellinek *et al.*, (1993, 1994) *supra*). Reverse transcription of the affinity-selected RNA and amplification by PCR were done as described previously (Jellinek *et al.*, (1994) *supra*). Nineteen rounds of SELEX were performed, typically selecting between 1-12% of the input RNA. For the first eight rounds of selection, suramin (3-15 µM) was included in the selection buffer to increase the selection pressure. The affinity-enriched pool (round 19) was cloned and sequenced as described (Schneider *et al.*, (1992) *supra*). Forty-six unique sequences have been identified, and the sequences are shown in Table 13 (SEQ ID NOS:128-170). The unique-sequence ligands were screened for their ability to bind PDGF AB with high affinity. While random 2'-fluoropyrimidine RNA (Table 12) bound to PDGF with a dissociation constant (Kd) of 35 ± 7 nM, many of the affinity-selected ligands bound to PDGF AB with ~100-fold higher affinities. Among the unique ligands, clones 9 (K<sub>d</sub> = 91 ± 16 pM), 11 (K<sub>d</sub> = 120 ± 21 pM), 16 (K<sub>d</sub> = 116 ± 34

pM), 23 ( $K_d = 173 \pm 38$  pM), 25 ( $K_d = 80 \pm 22$  pM), 37 ( $K_d = 97 \pm 29$  pM), 38 ( $K_d = 74 \pm 39$  pM), and 40 ( $K_d = 91 \pm 32$  pM) exhibited the highest affinity for PDGF AB (binding of all of these ligands to PDGF AB is biphasic and the  $K_d$  for the higher affinity binding component is given).

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#### EXAMPLE 16. EXPERIMENTAL PROCEDURES

This Example provides the general procedures followed and incorporated in Examples 17-19 for the evolution of nucleic acid ligands to hKGF.

##### A. Materials and Methods

- 10 Recombinant human Keratinocyte Growth Factor (hKGF) and human Epidermal Growth Factor (hEGF) were purchased from Upstate Biotechnology Inc.(Lake Placid, NY). haFGF, hbFGF, PDGF-AB, TGF $\beta$ 1, and anti-KGF neutralizing monoclonal antibody were purchased from R&D Systems (Minneapolis, MN). Recombinant rat KGF was purchased from QED Advanced Research Technologies (San Diego, CA). Human thrombin was purchased from Enzyme Research Laboratories (South Bend, IN). T4 DNA ligase, *Hpa*I methylase, and restriction enzymes were purchased from New England Biolabs (Beverly, MA). pCR-Script Amp SK(+) cloning kit was purchased from Stratagene (La Jolla, CA). AMV reverse transcriptase was purchased from Life Sciences (St. Petersburg, FL). Taq DNA polymerase was purchased from Perkin Elmer (Foster City, CA). Ultrapure nucleotide triphosphates were purchased from Pharmacia (Piscataway, NJ).  $\alpha$ - $^{32}$ P-ATP,  $\gamma$ - $^{32}$ P-ATP, and  $5'$  $^{32}$ P-cytidine 3', 5'-bis (phosphate) ( $5'$  $^{32}$ P-pCp) were from DuPont NEN Research Products (Boston, MA).  $^{125}$ I-labeled KGF was prepared as described before (Bottaro *et al.*, (1990) *J.Biol.Chem.* 265:12767-12770). PC-3 prostatic carcinoma cells were obtained from ATCC (catalog number CRL1435). Balb/MK cells and NIH3T3 transfected cells with the human KGF receptor (NIH3T3/KGFR) were a generous gift from S. Aaronson, Mt. Sinai Medical Center, NY, and have been described elsewhere (Miki *et al.*, (1992) *Proc.Natl.Acad.Sci.USA* 89:246-250; Miki *et al.*, (1991) *Science* 251:72-75; Weissman *et al.*, (1983) *Cell* 32:599-606). T7 RNA polymerase, 2'NH<sub>2</sub>- and 2'F-modified CTP and UTP were from NeXstar

Pharmaceuticals, Inc. (Boulder, CO). DNA oligonucleotides were obtained from Operon Technologies, Inc. (Alameda, CA). Nitrocellulose/cellulose acetate mixed matrix, 0.45 µm, HA filters were from Millipore (Bedford, MA). Calcium and magnesium containing Dulbecco's Phosphate Buffered Saline (DPBS) was purchased from Life Technologies (Gaithersburg, MD). Chemicals were at least reagent grade and purchased from commercial sources.

**B. SELEX**

The SELEX procedure has been described in detail in US patent 5,270,163 (see also Tuerk and Gold (1990) Science 249:505-510). A single-stranded DNA (ssDNA) pool was used to generate the double-stranded (dsDNA) template for generating the initial random sequence RNA pool by transcription. The DNA template contained 40 random nucleotides, flanked by 5' and 3' constant regions for primer annealing sites for PCR and cDNA synthesis (Table 14; SEQ ID NOS:186-188). The 5' primer contains the T7 promotor sequence for *in vitro* transcriptions. The template was PCR amplified following an initial denaturation at 93°C for 3.5 minutes through 15 cycles of 30 second denaturation at 93°C, 1 minute annealing at 60°C, and 1 minute elongation at 72°C, in 50 mM KCl, 10mM Tris-HCl, pH9, 0.1% Triton X-100, 3 mM MgCl<sub>2</sub>, 0.5 mM of each dATP, dCTP, dGTP, and dTTP, 0.1 units/µl Taq DNA polymerase, and 2.5 nM each of 3G7 and 5G7 primers (Table 14; SEQ ID NOS.187-188). SELEX experiments for hKGF were initiated with a random sequence pool of RNA in which all pyrimidines were 2'-NH<sub>2</sub>-modified or 2'-F-modified. Transcription reactions were done with about 5µM DNA template, 5 units/µl T7 RNA polymerase, 40mM Tris-HCl (pH8), 12 mM MgCl<sub>2</sub>, 5mM DTT, 1mM spermidine, 0.002% Triton X-100, 4% PEG 8000, 2-4 mM each 2'OH ATP, 2'OH GTP, 2'NH<sub>2</sub> or 2'F CTP, 2'NH<sub>2</sub> or 2'F UTP, and 0.25 µM α<sup>32</sup>P 2'OH ATP (800 Ci/mmol). The full length transcripts were gel-purified prior to use. To prepare binding reactions, the RNA molecules were incubated with recombinant hKGF in Dulbecco's Phosphate-Buffered Saline (DPBS) with calcium and magnesium (Life Technologies, Gaithersburg, MD, Cat. No 21300-025) containing 0.01% human serum albumin. Following incubation at room temperature (ranging from 10

minutes to 10 hours) the protein-RNA complexes were partitioned from unbound RNA by filtering through nitrocellulose. Nitrocellulose filter bound RNA was recovered by phenol/urea extraction. The partitioned RNA was reverse transcribed into cDNA by AMV reverse transcriptase at 48°C for 60 min in 50 mM Tris-HCl pH8.3, 60 mM NaCl, 6 mM Mg(OAc)<sub>2</sub>, 10mM DTT, 50 pmol DNA 3' primer (Table 14), 0.4 mM each of dATP, dCTP, dGTP, and dTTP, and 1 unit/ $\mu$ l AMV RT. The cDNA was PCR amplified and used to initiate the next SELEX cycle.

#### C. Nitrocellulose Filter Partitioning

In order to partition the protein-RNA complexes, the binding reactions were filtered through nitrocellulose/cellulose acetated mixed matrix, 0.45  $\mu$ m pore size (filter disks, Millipore, Co., Bedford, MA). For filtration, the filters were placed onto a vacuum manifold and wetted by aspirating 5 ml of DPBS. The binding reactions were aspirated through the filters, and following a 5 ml wash, the filters were counted in a scintillation counter (Beckmann). Higher wash volumes with DPBS or 0.5 M urea were used as a means to increase selection stringency as shown in Table 15. Gel purified, internally  $\alpha$ -<sup>32</sup>P-ATP labeled transcripts were incubated with various concentrations of hKGF in DPBS at 37°C for 10 minutes. Oligonucleotide protein mixtures were filtered through prewetted 0.45  $\mu$ m pore size HA filters, followed by a 5 ml wash with DPBS. The radioactivity retained on the filter was counted and corrected for background binding in the absence of protein. Nonlinear least square method was used to fit the data into monophasic or biphasic binding curves and to obtain the equilibrium dissociation constant  $K_d$  (Jellinek *et al.*, (1993) Proc.Natl.Acad.Sci. USA 90:11227-11231) using the software package Kaleidagraph (Synergy Software, Reading, PA). Biphasic binding can be described as the binding of two affinity species that are not in equilibrium.

#### D. Cloning and Sequencing

The RNA recovered from the round 8 filters was reverse transcribed and PCR amplified. Following column purification with QIA-quick spin columns (Qiagen, Inc., Chatsworth, CA) and ethanol precipitation, the amplified DNA was

methylated with HpaII methylase (New England Biolabs, Beverly, MA). The methylated DNA was cloned into the SrfI restriction site of pCR-Script Direct SK(+) plasmid using the pCR-Script Amp SK(+) cloning kit (Stratagene Cloning Systems, La Jolla, CA). About 80 clones were sequenced with Sequenase sequencing kit (United States Biochemical Corporation, Cleveland, OH). Sequence analysis and secondary structure prediction was done by using previously described computer software (Feng and Doolittle (1987) *J. Mol. Evol.* 25:351-360; Jaeger *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:7706-7710; Jaeger *et al.*, (1990) *Methods Enzymol.* 183:281-306; Zucker (1989) *Science* 244:48-52).

#### E. Determination of Minimal Sequences Necessary for Binding

Oligonucleotide ligands end labeled at the 5' end with  $\gamma$ -<sup>32</sup>P-ATP using T4 polynucleotide kinase, or at the 3' end with 5'-<sup>32</sup>P-pCp and T4 RNA ligase, were used to establish 3' and 5' boundaries respectively (Fitzwater *et al.*, (1996) *Methods Enzymol.* 267:275-301). After partial alkaline hydrolysis, the radiolabeled oligonucleotide was incubated with 0.1, 0.6, and 3.0 nM hKGF, and the protein bound oligonucleotide was isolated by nitrocellulose filtration. The nitrocellulose retained oligonucleotide truncates were analyzed on a high resolution denaturing polyacrylamide gel. An alkaline hydrolysis ladder and a ladder of radioactively labeled ligands terminated with G-residues, generated by partial RNase T1 digestion, were used as markers to map the 3' and 5' boundaries.

#### F. Thermal Denaturation Profiles

Oligonucleotide melting profiles were obtained with a Cary Model 1E spectrophotometer. Oligonucleotides were heated to 95°C in PBS (Sambrook *et al.*, (1989) *Molecular Cloning: A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY) or 10 mM phosphate buffer and cooled to room temperature before recording the melting profile. The melting profiles generated show the change in absorbance at 260 nm as a function of temperature. During recording, the samples were heated at a rate of 1°C min<sup>-1</sup> from 20-95°C.

EXAMPLE 17. RNA LIGANDS TO hKGFA. SELEX

To generate RNA ligands for hKGF, two parallel SELEX experiments were initiated, one with 2'-NH<sub>2</sub> and the other 2'-F pyrimidine modified RNA molecules randomized at 40 contiguous positions. The SELEX conditions and results for each round are summarized in Table 15. The starting pool contained 5x10<sup>14</sup> (500 pmoles) and 2.5x10<sup>14</sup> (250 pmoles) 2'-NH<sub>2</sub> and 2'-F pyrimidine modified RNA molecules, respectively, and bound to hKGF with an approximate  $K_D$  of 30 nM. After 8 rounds of SELEX, the evolved pools bound with a  $K_D$  of 0.6 nM. No further improvement in the  $K_D$  was observed in the subsequent two rounds. The RNA pools from the 8th round were reverse transcribed, PCR amplified and cloned as described.

B. RNA sequences

In the 2'-NH<sub>2</sub> SELEX, 29 out of 31 clones were unique. In the 2'-F SELEX all 43 clones sequenced were unique. A unique sequence is defined as one that differs from all others by three or more nucleotides. Table 16 lists the sequences (SEQ ID NOS:189-262) of all of the clones sequenced in standard single letter code (Cornish-Bowden, (1985) *Nucleic Acid Res* 13:3021-3030). Computer assisted global and local alignment did not reveal any extensive homologies among the clones, and no obvious families were apparent. The 2'-NH<sub>2</sub> clones are in general purine rich while the 2'-F clones are pyrimidine rich. When the alignment parameters were relaxed, the Feng/Doolittle algorithm grouped the 2'-NH<sub>2</sub> clones in one family and the 2'-F clones in another. Visual inspection of the sequences suggested two and three possible families for the 2'-NH<sub>2</sub> and the 2'-F ligands, respectively. Using conserved predicted secondary structure, 38 2'F ligands could be assigned into two classes (Figures 12A and 12B). Similarly, 15 2'NH<sub>2</sub> ligands could be assigned into two classes (Figures 12C and 12D). The two proposed classes for the 2'F ligands can be folded into pseudoknot structures (Wyatt *et al.*, (1993) *The RNA World* 465-496; ten Dam, E. (1992) *Biochemistry* 31:1665-1676). These structures are very related and in fact they could be circular permutations of a common structure. Loop 3 (L3) of class 1

pseudoknots presents the conserved sequence 5'RRYuy while loop 1 (L1) of class 2 ligands presents the sequence 5'AaYY. Both of these sequences contain the consensus 5'RRYY. Some of the 2'F ligands contain two to three copies of the RRYY sequence (Figures 12A and 12B). Another feature of these structures is the unequal distribution of purines and pyrimidines in stem 1 (S1). One strand of that stem contains almost exclusively purines while the other strand contains pyrimidines.

Class 1 of the 2'NH<sub>2</sub> ligands includes 8 members that can be folded into stem-loop structures with internal symmetric or asymmetric loops. The stem contains three consecutive GC base pairs. The terminal loops are long and present the conserved sequence 5'GGAA(N)<sub>1-14</sub>YAA(N)<sub>1-14</sub>RCRR (SEQ ID NO:263). Both sides of the internal asymmetric loops of the class 1 ligands contain the sequence 5'AA. Class 2 includes 7 ligands that can be folded into dumbbells with variable sized loops. One loop contains the conserved sequence 5'YGAY while the other loop contains the conserved sequence 5'GGAA(N)<sub>0-4</sub>YGA (SEQ ID NO:264). Clones 2N and 54N are circular permutations of the remaining 5 clones.

### C. Affinities

The dissociation constants of the hKGF ligands were determined by nitrocellulose filter binding and are listed in Table 17. Eight out of 41 2'-F ligands bound biphasically. The remaining of the 2'-F and all the 2'-NH<sub>2</sub> ligands bound monophastically. Under protein excess, biphasic binding suggests that the ligand exists as two affinity species (presumably isoconformers) that are not in equilibrium. The best 2'-F-modified ligand, K14F, binds biphasically with the high and low affinity dissociation constant at about 0.3-3pM and 2-10 nM respectively. There is some observed variability in the  $K_D$  determinations for the various clones and the random RNA. Despite the experimental variability in the  $K_D$  determinations, the high affinity species of K14F have a 1,000-5,000 fold better affinity than the random RNA. Among the monophasic 2'-F-modified ligands, K38F had the best KD of about 0.3nM. The best 2'-NH<sub>2</sub>-modified ligands bound with a KD of 0.4nM which represent about 75 fold improvement over the random RNA.

#### **D. Determination of Minimal Sequences Necessary for Binding**

Two 2'F ligands (6F and 14F) (SEQ ID NOS:223 and 231) were studied further to determine the minimal sequences necessary for binding. Sequence boundaries were determined by allowing an alkaline hydrolysis ladder, labeled at the 3' or 5' end, to bind to hKGF. The partial fragments were affinity purified by nitrocellulose filtration and analyzed on high resolution denaturing gels. Boundaries were clearly observed only at the 3' ends for both ligands (Figure 13) and are in agreement with the class 1 proposed folding as shown in Figures 12A and 12B. Truncated templates were then used to confirm the boundaries (Figure 13). Three truncates were tested for 6F because a run of 7 consecutive pyrimidines did not allow the precise mapping of the boundary. From these three truncates, one lost its KGF binding activity as shown in Figure 13. A single 14F truncate, designated 14F3'T, was tested. This truncate was two bases longer than the observed boundary in order to extend stem 2 (S2) of the proposed pseudoknot structure. The 14F3'T truncated ligand retained binding activity with affinity similar to the full length ligand. Like the full length ligand, 14F3'T bound KGF biphasically where the high affinity species represented about 20% of the molecules and showed  $K_d$  values of about 0.3-3 pM. These high affinity species when partially separated from the low affinity species on the basis of differential affinity to KGF, exhibited binding curves with mid points at 0.3-3 pM and maximum plateaus of about 70% (data not shown). Figure 13 shows the predicted folding of the shortest active truncates for 6F and 14F which are 53 and 49 bases long respectively. Both proposed pseudoknot structures contain relatively long stems. The two proposed stems of 6F are separated by a single base forming a non-H-type pseudoknot. The proposed 6F structure resembles the solution structure of a similar pseudoknot motif from a frame-shifting element found in the MMTV RNA (Shen *et al.*, (1995) *J.Mol.Biol.* **247**:963-978). The two stems (S1 and S2) of 14F could be drawn as two coaxially stacked helices of 16 base pairs total length (H-type pseudoknot). A Similar pseudoknot structure has been proposed before, based on NMR data (Du *et al.*, (1996) *Biochemistry* **35**:4187-4198). Given the short length of L1, it is possible that ligand 14F forms a

non-H-type pseudoknot where the last GU base pair of S1 is not formed allowing a more flexible helical region and a longer L1. Temperature melting curves of 14F and 14F3'T suggest a remarkable thermostability for this ligand (data not shown). These melting curves appear to be concentration independent and biphasic in 150 mM salt. Biphasic melting curves have been observed before with tRNA (Hilbers *et al.*, (1976) *Biochemistry* 15:1874-1882), and have been attributed to the tertiary folding of the RNA molecule. Multiphasic temperature transitions have also been proposed for RNA pseudoknots (Du *et al.*, (1996) *Biochemistry* 35:4187-4198). The biphasic curves observed include a low Tm at about 55°C and a high Tm of greater than 85-90°C. In 10 mM salt the low Tm of 14F is not observed while the high Tm is shifted down to 75-78°C. The melting profile for 14F appears to be flatter than 14F3'T even though the Tm values are the same. The data suggest that the observed thermostability is attributable to just the minimal 49-mer.

In an effort to identify shorter KGF ligands that retained binding, the binding activity of various deletions of the shortest truncate of ligand 14F, namely 14F3'T were tested. Deletions were tested in all the structural elements of the proposed pseudoknot structure. The results are summarized in Table 23 (SEQ ID NOS:272-304). RNA transcripts containing 2'F pyrimidines and 2'OH purines were obtained by in vitro transcription using synthetic DNA templates. The activity of each ligand is shown by scoring for both the high (H) and low (L) affinity component of the 14F3'T binding curve with + (active) or - (not active). Truncates T35 and T36 represent two complementary halves of 14F3'T molecule and were additionally tested as an equimolar mixture. The structural elements of the proposed pseudoknot structure are separated by (|) and are indicated by symbols S1 (stem 1), S2 (stem 2), L1 (loop 1) and L3 (loop 3). The proposed pseudoknot structure for 14F3'T is a non-H-type pseudoknot and lacks L2 (loop 2). The complementary sequences forming S1 (S1 and S1') and S2 (S2 and S2') are marked by single and double underlines respectfully. In the tabulated sequences, deleted bases were replaced with periods (.). Any deletion attempt in the stems S1 and S2 of the proposed pseudoknot structure resulted in loss of both

the high (H) and low (L) affinity component of the binding curve as observed with the 14F3'T ligand. Deletions in loop 3 (L3), however, were tolerated as long as one copy of the RRYY box remained intact. The shortest ligand that retained activity is T22 which is a 43-mer. In trying to obtain shorter ligands by truncating L3 further a mutant version of T22 (designated T22mu) was used where the last GC base pair of S1 was eliminated by a G to U mutation at position 6. The reasoning for this mutation was to enhance the flexibility of the double stranded region of this ligand by allowing an unpaired base between S1 and S2. Although this mutation did not affect the binding of T22 it did not allow further active truncations in L3.

#### E. Specificity of RNA Ligands to hKGF

The specificity of the K14F ligand was tested by determining its  $K_D$  against rat hKGF, and the heparin binding human growth factors, aFGF, bFGF, and PDGF (Table 18). The results suggest that the K14F binds all tested targets like random RNA, except hKGF, and it can discriminate between hKGF and other similar proteins by a factor of 400-40,000.

The specificity of ligand 14F3'T was tested by determining its  $K_d$  against a variety of heparin binding proteins. The results summarized in Table 22 show that ligand 14F3'T can discriminate KGF from all other heparin binding proteins tested by a factor of  $1.2 \times 10^4$ - $3 \times 10^{10}$ . Ligand 14F3'T binds only to KGF with high affinity while it binds all other heparin binding proteins tested like random RNA. Binding of 14F3'T to the rat KGF, which is 91% identical to human KGF, is with about a 5-10 fold reduced affinity. Similar specificity was observed during the inhibition of the KGF induced DNA synthesis of Balb/MK cells. Ligand 14F3'T inhibits rat KGF induced DNA synthesis with a  $K_i$  of 1.8 nM which is 20-50 fold higher than the  $K_i$  observed with the human KGF. Ligand 14F3'T inhibits the DNA synthesis of Balb/MK cells only if it is the result of KGF but not EGF stimulation (data not shown).

**EXAMPLE 18. INHIBITION OF hKGF BINDING TO CELL SURFACE RECEPTORS**

**A. Receptor Binding Assay**

5 To test the ability of the hKGF ligands to competitively inhibit the binding of hKGF to its cell surface receptor, two cell lines were used. The first cell line, PC-3, is an isolate from a grade IV prostatic adenocarcinoma (ATCC CRL 1435). The second cell line is designated as NIH3T3/FGFR-2 and is a recombinant NIH/3T3 cell line carrying the human hKGF receptor at about 0.5-1X10<sup>6</sup> high affinity KGF binding sites per cell (Miki *et al.*, (1992) Proc. Natl. Acad. Sci. USA 89:246-250).

10

PC-3 cells were plated in 24-well plates at about 10<sup>5</sup> cells per well. Following growth for 48-36 hours, the cells were serum starved for 24 hours, washed two times with 500 µl of cold DPBS, and then incubated with 500 µl 15 binding buffer (BB1; DPBS, 0.5mM MgCl<sub>2</sub>, 0.2% BSA, 0.02% sodium azide) containing various concentrations of <sup>125</sup>I-labeled KGF ranging from 0 to 0.8 nM. Following 3-3.5 hour incubation at 4°C, the binding mixes were aspirated and the well-adhered cells were washed two times with 1 ml BB1 and once with 1 ml BB1 supplemented with 0.5M NaCl. The remaining bound labeled hKGF was 20 solubilized in 600 µl 0.5% SDS/0.1M NaOH and counted in a gamma counter (Beckmann). Nonspecific binding was determined in the presence of 100 fold molar excess of unlabeled hKGF. For competition assays, the labeled hKGF was kept constant at 0.3 nM, and varying concentrations of competitor molecules were included in the binding reactions ranging from 0 -1,000 nM. Binding curves were 25 fitted to the equation:

$$[\text{Bound Tracer}] = ([\text{Total Tracer}] * [\text{Receptor}]) / (K_D + [\text{Total Tracer}])$$

where [Total Tracer] and [Bound Tracer] were fixed and the K<sub>D</sub> and [Receptor]

30 were determined by regression analysis using the software Kaleidagraph (Synergy Software, Reading, PA).

NIH3T3/KGFR-2 cells were plated in 24-well plates at about  $10^5$  cells per well. Following growth overnight, the cells were serum starved for 1-5 hours, washed two times with 500  $\mu$ l binding buffer (BB2: serum-free MEM growth medium, 0.1% BSA, 25mM HEPES, pH 7.4), and then incubated with 250  $\mu$ l BB2 containing 1  $\mu$ g/ml heparin (from bovine lung, SIGMA, St. Louis, MO),  
5  $^{125}$ I-labeled hKGF at 0.03 nM, and varying concentrations of competitor molecules (300nM-0nM). Following 1 hour incubation at room temperature, the binding mixes were aspirated, and the wells were washed two times with 250  $\mu$ l cold DPBS and once with 250  $\mu$ l cold DPBS supplemented with 0.5M NaCl. The  
10 bound labeled hKGF was solubilized in 500  $\mu$ l 0.5% SDS and counted in a scintillation counter (Beckmann).

The inhibition constants ( $K_i$ ) of the RNA ligands were determined by a nonlinear regression analysis of the data.

In search of KGF receptors on the surface of PC-3 cells, different  
15 concentrations of  $^{125}$ I-hKGF were used, ranging from 0.002 to 0.8 nM, in the presence and absence of 100 fold molar excess of unlabeled hKGF, and saturation binding of the tracer on the surface of PC-3 cells was observed. Figure 10 shows the plot of the concentration of bound tracer as a function of the total concentration of tracer as well as the Scatchard analysis of the same data.  
20 Analysis of the data suggested that there are about 5,000 specific hKGF binding sites per cell with a  $K_D$  of 100-200 pM. This  $K_D$  is in good agreement with the reported  $K_D$  for hKGF of 200 pM (Miki *et al.*, (1992) Proc natl Acad Sci USA 89:246-250).

PC-3 plasma membrane extracts were found to alter the electrophoretic mobility (gel shift) of radiolabeled hKGF upon native gel electrophoresis (Figure 11). For electrophoretic mobility shift gels, about  $3 \times 10^7$  PC-3 cells were gently spun and washed with PBS and then lysed by mixing with equal volume of lysis buffer containing 40 mM Hepes, pH 7.4, 150 mM NaCl, 20% glycerol, 2% triton X-100, 0.1% sodium azide, 3 mM MgCl<sub>2</sub>, 3 mM EGTA, 2  $\mu$ M aprotinin, 2  $\mu$ M leupeptin, 2 mM PMSF, and 400  $\mu$ M sodium orthovanadate. Following 15 min incubation on ice the extract was spun at 11,000 g at 4°C for 30 min to remove  
25  
30

debris and nuclei and the supernatant was aliquoted and stored at -70°C. For gel analysis, 25 $\mu$ l binding reactions were set in DPBS, 0.01% HSA, 2 mM MgCl<sub>2</sub>, containing 3  $\mu$ l of a 10 fold diluted PC-3 membrane extract in 0.01% HSA, and various concentrations of <sup>125</sup>I-labeled hKGF. Following a 10 min incubation at room temperature, 6X loading dye was added to achieve 1X concentration, and the samples were loaded onto a 5% or 10% native TBE polyacrylamide gel. The gel was prerun at room temperature at 100 Volts. Following loading, the gel was run at 200 Volts for 5 min and then at 100 Volts for 30-60 min at room temperature. The radioactive bands were then visualized by autoradiography. The gel shift of radiolabeled hKGF is not observed in the presence of 100 fold molar excess of unlabeled hKGF (Figure 11), demonstrating a specific interaction between a component found in the PC-3 membrane extracts and hKGF. The estimated KD from the gel shift experiment is about 8 nM.

In agreement with the competition experiments reported in the literature (Miki *et al.*, *Proc Natl Acad Sci USA* 89:246-250), gel shift competition curves using unlabeled hKGF and bFGF as well as an unrelated small basic protein namely lysozyme were obtained. Table 21 lists the IC50 values obtained in this experiment. In agreement with previous reports, the data presented in Table 21 show that bFGF competes about 20 fold worse than hKGF for binding with the hKGF receptor present in the PC-3 plasma membrane extracts. The interaction observed by the gel shift appears to be a specific interaction for FGF and it is not due to a charge-charge interaction, as lysozyme, another small positively charged molecule, competes for the PC-3 membrane extract:hKGF complex with about 100 fold worse affinity than hKGF alone.

IC50 values for various RNA ligands obtained with the PC-3 assay are shown in Table 19. A subset of these ligands was tested on the NIH3T3/FGFR-2. Competitive inhibition constants (Ki) were determined from full competition curves and are summarized in Table 20. In determining the Ki values, it was assumed that 3T3 cells have 500,000 binding sites per cell and PC-3 cells have 5,000 binding sites per cell.

The data show that several hKGF ligands can competitively inhibit binding of hKGF to its cell surface receptors. Some of these ligands, such as K14F, have potent competitive activities with  $K_i$ 's in the low nM range.

5 This work not only demonstrates that nucleic acid competitors for hKGF were obtained, but also identifies a new assay for screening hKGF competitors including small molecules, antibodies, and peptides. This new assay includes the use of the prostate carcinoma cell line, PC-3.

10 The two cell lines, PC3 and NIH3T3/FGFR-2, give slightly different results (see Table 20). KGF binding to PC-3 cells is more sensitive to inhibition by several ligands and by heparin. Random RNA, however, does not effectively compete for KGF binding on the PC-3 cells. KGF binding to NIH3T3/FGFR-2 is resistant to inhibition by some RNA ligands and heparin. This is because the NIH3T3/KGFR assay is more stringent since it is done in the presence of 1  $\mu$ g/ml heparin. The random oligonucleotide competition curve with the  
15 NIH3T3/FGFR-2 is completely flat with  $K_i > 10^4$  M. Ligands 6F and 14F show the best inhibitory activity with  $K_i$  values of 100-200 pM and 2-8 nM in the PC-3 and NIH3T3/FGFR-2 assay respectively. Only two 2'NH<sub>2</sub> ligands, 14N and 29N, show good activity with the PC-3 cells ( $K_i$  value of 1.4 nM). From these two ligands, only 14N retains its inhibitory activity in the NIH3T3/FGFR-2 assay showing a  $K_i$  value of 100 nM. The observed inhibition of the KGF mitogenic activity by these ligands is not due to a nonspecific affect in the proliferative ability of the cell lines because these ligands have no antiproliferative activity on cells induced by EGF instead of KGF (data not shown).

20

25 This work not only demonstrates that nucleic acid competitors for hKGF were obtained, but also identifies a new assay for screening hKGF competitors including small molecules, antibodies, and peptides. This new assay includes the use of the prostate carcinoma cell line, PC-3.

**EXAMPLE 19. INHIBITION OF THE MITOGENIC ACTIVITY OF KGF**

One of the biological effects of KGF is the stimulation of proliferation of epithelial cells (Rubin *et al.*, (1989) *Proc Natl Acad Sci USA* **86**:802-806). This proliferative effect of KGF can be measured by the stimulation of  $^3\text{H}$ -thymidine incorporation in responding cells after exposure to KGF. Three such cell lines have been described before (Rubin *et al.*, (1989) *Proc Natl Acad Sci USA* **86**:802-806). Two cell lines were used to test the anti-mitogenic activity of various ligands. One is 4MBr-5 (ATCC #CCL208), a monkey epithelial, low passage, cell line (Caputo *et al.*, (1979) *In Vitro* **15**:222-223) while the second is Balb/MK, a transformed rat keratinocyte cell line (Weissman and Aaronson (1983) *Cell* **32**:599-606). 4-MBr5 cells grown in F12K containing 30 ng/ml, hEGF, and 10% FCS, were trypsinized and resuspended in M199 containing 10 mM HEPES, pH 7.4, and 10 % FCS at  $1.4 \times 10^5$  cells/ml. A 96-well microtiter plate was seeded with 100  $\mu\text{l}$  of cell suspension and KGF was added at 10 ng/ml (0.5 nM), as well as K14F ligand at various concentrations ranging from 0-1000 nM. Each incubation reaction was set in at least triplicates. Following 24 h incubation at 37°C,  $^3\text{H}$ -thymidine was added at 1  $\mu\text{Ci}/\text{well}$  along with unlabeled thymidine at 10 nM. The cells were incubated for additional 24 h, the supernatant was aspirated, and the remaining cells were harvested by lysis in 20  $\mu\text{l}$  of 0.2 N NaOH. The extent of  $^3\text{H}$ -thymidine incorporation was determined by TCA precipitation and filtration through GFC filter disks (Whatman, Hillsboro, OR).

Balb/MK cells grown in Low Ca<sup>++</sup> EMEM with 10% FCS (dialyzed and heat inactivated) and 5 ng/ml rhEGF were trypsinized and resuspended in Low Ca<sup>++</sup> EMEM with 1% FCS (dialyzed and heat inactivated) and 0.5 ng/ml rhEGF and plated on 96 well fibronectin coated culture plates at  $4-6 \times 10^4$  cells per well in 100  $\mu\text{l}$  total volume. Following overnight growth, the medium was replaced with Low Ca<sup>++</sup> EMEM without FCS or rhEGF and serum starved for about 30 hrs. Human recombinant KGF or EGF was then added at 16 and 49 pM respectively, along with various concentrations of competitors ranging from 0-1000 nM. Following over-night incubation,  $^3\text{H}$ -thymidine was added at 0.2  $\mu\text{Ci}/\text{well}$  and incubation continued for an additional 7-8 hrs. The extent of  $^3\text{H}$ -thymidine

incorporation was determined by TCA precipitation and filtration through GFC filter disks.

The inhibition constants ( $K_i$ ) of the oligonucleotide ligands were determined by a nonlinear regression analysis of the data as described before (Gill et al., (1991) *J.Mol.Biol.* 220:307-324).

The two assays give slightly different results. The 4MBr-5 assay was performed in the presence of fetal calf serum, while the Balb/MK was done following serum starvation. The Balb/MK assay is more sensitive and a prototypic assay for the KGF induced mitogenic activity. Similar to the results obtained with the PC-3 cells, the 4MBr-5 assay showed a good activity for ligand 14F ( $K_i$  value of 9.8 nM but incomplete inhibition). In the same assay, the random oligonucleotides showed  $K_i$  values of  $>1\mu M$  while a monoclonal neutralizing antibody showed a  $K_i$  value of 2.9 nM. It appears that ligand 14F is as good or even better than the monoclonal neutralizing antibody. The competition curves for the neutralizing monoclonal antibody and ligand 14N plateau at about 20-40%, suggesting that these antagonists do not completely abolish the KGF mitogenic activity. In contrast to the monoclonal antibody, ligand 14F completely blocks the KGF mitogenic activity on the 4MBr-5 cells. In the Balb/MK assay, 14N showed  $K_i$  values of about 10 nM (incomplete inhibition) while the random oligonucleotide showed  $K_i$  values of about 300 nM. The  $K_i$  values for 6F and 14F are 830 and 92 pM, respectively. Similar to the 4MBr-5 assay, ligand 14F appears to be as good if not better than the monoclonal neutralizing antibody which shows a  $K_i$  value of 980 pM. The best inhibitory activity was observed with 14F3'T with a  $K_i$  value of 34 pM.

#### **EXAMPLE 20.**

Nucleic acid ligands that bind to basic fibroblast growth factor (bFGF) have been derived by the SELEX method as described in U.S. Patent No. 5,459,015 (see also U.S. Patent No. 5,270,163 and Tuerk and Gold (1990) *Science* 249:505-510). A 2'NH<sub>2</sub>-modified nucleic acid ligand designated 21A having the sequence 5'-

GGGAGACAAGAAUAACGCUCAGUAGACUAAUGUGUGGAAGACAGC  
GGGUGGUUCGACAGGAGGCUCACACAGGC (SEQ ID NO:265) was  
examined by deletion analysis for the minimal sequence information required for  
high affinity binding to bFGF. This analysis led to truncated ligand 21A-t  
5 (GGUGUGUGGAAGACAGCGGGUGGUuc (SEQ ID NO:266) where the  
underlined G's are guanines added to improve efficiency of transcription and  
lowercase letters are from the constant region.

In order to increase the stability of ligand 21A-t against degradation by  
nucleases, short phosphorothioate caps were added to the 5' and the 3' ends. In  
10 addition, nine ribopurine positions were identified that can be substituted with 2'-  
deoxy-2'-O-methylpurines without a loss in binding affinity for bFGF, using the  
method described in Green *et al.*, *Chem.Biol.* 2:683-695, resulting in the ligand  
designated as NX-286 (5'-TsTsTsTs mGmGaU rGaUrG aUrGrG mArArG  
15 mAaCrA rGaCmG mGmGaU mGmGaU aUaC TsTsTsTsT-3' (SEQ ID NO:267),  
where s represents phosphorothioate internucleoside linkage, aU and aC are 2'-  
deoxy-2'-aminouridine and 2'-deoxy-2'-aminocytidine residues, respectively, mA  
and mG are 2'-deoxy-2'-O-methyladenosine and guanosine residues, respectively,  
rA and rG are adenose and guanosine residues and T is 2'-deoxythymidine). The  
modified nucleic acid ligand had a  $K_d$  of 0.4 nM as measured by electrophoretic  
20 mobility shift assay.

Table 1

## Nucleic Acid Sequences Used in SELEX Experiments described in Examples 1-4

\*GAUC or GATC, these bases only  
gauc or gact 62.5 % specified base, 12.5 % the other three bases

Table 2

**RNA SELEX Experiments described in Examples 1-4: template, pyrimidine nucleotides, and round cloned.**

<u>SELEX exp</u>	<u>template*</u>	<u>2'substituted UTP</u>	<u>2'substituted CTP</u>	<u>Round cloned</u>
lib1	64N6	OH	OH	20
lib2	64N6	OH	OH	6
lib3	40N6+64N6	F	F	4
lib4	40N6+64N6	NH <sub>2</sub>	NH <sub>2</sub>	5
lib5	64N6	NH <sub>2</sub>	NH <sub>2</sub>	13
lib6	64N6	F	F	13
lib7	64N6	F	NH <sub>2</sub>	14
lib8	D-123	OH	OH	6
lib9	64N6	NH <sub>2</sub>	F	5

\* Sequences of templates are described in Table 1.

Table 3  
TGF $\beta$  Binding ligands

SEQ ID NO.	clone	5' CONSTANT	VARIABLE	3' CONSTANT
1ib3	Group A	ggggagaacgcggaucc	[ 40 or 64 N]	aacuuuccucuagaucuuuauugaggguua
13	GAGCAAUCCCAAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGGG			12
3	GAGCAACCCCAAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG			13
4	GAGCAACCCCAAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG			14
1ib4				15
32	GCAACCCCAAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG			
1ib8				16
9	GCAAUCCAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG			17
1ib5				18
5	GAGCAAUCCCAAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGG			19
7	GAGCAAGGCCUGGC	AUAGCUCUCCGAGUAGACAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG		
48	G	GCAAUCCCAAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG		
1ib2				20
		6 - 4GAGCAAUCCCAAGGGCAUAGCUCUCCGAGUAGACAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG		
1ib6				21
23	A	AGCUUC GAGUAGACAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG		22
4	GAGCAUCCUA	GCAUAGCUUC GAGUAGACAGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG		
1ib7				23
21	GAGCAAUCCCGGGCGCAUAGCUCUCCGAGGAGACAGGGGGAGGGGGGAUUGGGGGGAUUCGGGUUCUACUGGGGG			24
43	GAGCAAUCCCAAGGGCAUAGCUCUCCGAGGAGACAGGGGGAGGGGGGAUUCGGGUUCUACUGGGGG			

Table 3 (Page 2)

clone	5' CONSTANT	VARIABLE	3' CONSTANT	SEQ ID NO.
<b>clone# 9999gagaacggggaucc [40 or 64N] aaggcuuucgcucuagaucuccuuuauugaggguua</b>				
<b>Group B.</b>				
1ib1-12	UGAGAAGGGACGUCCCCGUACGGGGGAGGGUGCAGCGA		AAGGGCCGGCACCAUGACGUAA	28
1ib3-44	UGAGAAGGGACGUCCCCU	GAGGGCGAGGAGA	AGGGCCGGCACCAUGACGUAA	29
1ib3-42	GGGGGAAA GUCCGAUU	AUGGUU GUAGAUUU GU GUGCGA		30
<b>Group C.</b>				
1ib1-20-31**	UGCUUAGACCGGAGAAUGCAAAAGGGACAUGCAUAGGGAAACCTUAUGUUAUARGAACG3CGGUUCGCAG			32
1ib1-20-31**	UGCUUAGACCGGAGAAUGCAAAAGGGACAUGCAUUAUGGGAAACCTUAUGUUAUAGAACCGGGGUUCGCAGA			33
1ib6-30**	UGCUUAGACCGGAGAAUGCAAAAGGGACAUGCAUUAUGGGAAACCUAU UAUAGAACGCCGUUCGCAG			34
<b>Group D.</b>				
1ib2-6-1*	UGUCUCCACCGGGAAUACUGGGGUUCUGGGGCCCUCCAUUGCAAGAGGGGGGGGUUCGGAGA			35
1ib2-6-1-81*	UGUCUCCACCGGGAAUACUGGGGUUCUGGGGUUCUGGGGUUCUGGGGUUCUGGGGUUCUGGGGUUCGGAGA			36
1ib8-23*	UGUCUCCACCGGGAAUACUGGGGUUCUGGGGUUCUGGGGUUCUGGGGUUCUGGGGUUCGGAGA			37
1ib9-10*	UGUCUCCACCGGGAAUACUGGGGUUCUGGGGUUCUGGGGUUCGGAGA			38
<b>ORPHANS.</b>				
<b>clone#</b>				
1ib3-45	GGAAAGUCUGGUUCUUTUGGGAGUCGCCGAUGGCCUGGGGA			39
1ib1-20-5**	AACGAGUGGUUCGGCGCACGAGGUACUGGGGACAGGGGUACGGGACGGGUUTUGGCC			40
1ib1-20-12**	GGUCACCCGGCAUUAACAUAGCCGACACUGGGGUACUGGGACUGGACCGGGAC			41
1ib2-6-8***	AUAACCCGGUGCAUGGGAGGGACAUCCUGGGAAAGGACGGGUCAUGGACCUAGGACGUAGGACGUCCGGC			42

Table 3 (Page 3)

clone	SEQ ID
<b>Group A Boundary Experiments</b>	
lib3 - 13 boundaries 5' GCUUCCGAGUAGACAGGAGGGGGGGGAUC 3'	25
lib8 - 9 boundaries 5' CUCCGAGUAGACAGGAGGGGGGGGAUC 3'	26
lib4 - 32 boundary GGCACACCCCAGGGCAUAGCTTCGAGUAGACAGGGGGGGGGGAUC 3'	27
<b>Group B Boundary Experiments</b>	
lib4 - 12 boundaries 5' UGAGAAGGGACGUCCCCGUCAACGGGUGGGAGGCAGCAGAANGGGGGCCACCA 3'	31

Legend: The constant region of the ligand is shown in lower case and variable in upper. Sequences have been aligned. Deletions with respect to the first sequence in each group are shown by gaps, substitutions are in bold type.

\*2'NH12-UTP, 2'F-CTP;

\*\*2'F-UTP, 2'F-CTP;

\*\*\*2OH-UTP, 2OH-CTP

Group A and B bind with either 2'NH12- or 2'F- pyrimidines.

Ligands bind with either 2'NH12- or 2'F- pyrimidines unless otherwise indicated.

**Table 4**  
**Dissociation and Inhibition Constants**

Group	Ligand	B <sub>max</sub>	K <sub>d</sub>	I <sub>C</sub> <sub>50</sub>
A	lib3-13	0.60	0.9 nM	9.7 nM
		0.38	0.7 nM	42 nM
		0.55	0.9 nM	18 nM
				32 nM
	lib3-3	0.44	1.7 nM	NT
	lib4-32	0.50	0.8 nM	20 nM
				157 nM
	lib5-5	0.37	2.4 nM	49 nM
	lib5-7	0.33	3.4 nM	17 nM
	lib8-9	0.4	1.7 nM	210 nM
B	lib8-9*	0.35	2.8 nM	124 nM
	lib5-48	0.32	3.8 nM	not inhibitory
	lib2-6-4	0.20	3.1 nM	not inhibitory
	lib6-23	0.35	3.4 nM	not inhibitory
	lib7-21****	0.18	2.4 nM	not inhibitory
	lib7-43****	0.33	3.3 nM	not inhibitory
	lib4-12	0.15	0.4 nM	109 nM
C		0.08	0.2 nM	108 nM
				69 nM
D	lib3-44	0.18	1.3 nM	119 nM
	lib3-42	0.16	0.6 nM	22 nM
Orphans	lib1-20-3**	0.67	30 nM	not inhibitory
	lib1-20-3-82**	0.46	6.1 nM	not inhibitory
	lib6-30**	0.35	8.8 nM	not inhibitory
Controls	lib2-6-1*	0.40	14.3 nM	112 nM
				103 nM
	lib2-6-1-81*	0.39	10.7 nM	201 nM
				298 nM
Orphans	lib8-23*	0.48	6.6 nM	not inhibitory
	lib9-10*	0.24	1.1 nM	not inhibitory
	lib3-45	0.08	1.9 nM	not inhibitory
	lib1-20-5**	0.42	46 nM	not inhibitory
Controls	lib1-20-12***	0.34	3.1 nM	NT
	lib1-6-8***	0.12	4.7 nM	NT
	lib5-9		nonbinder	not inhibitory
	random 64N6		nonbinder	not inhibitory

ligands are 2'- NH2 pyrimidines unless otherwise noted

\* 2'-NH2-UTP, 2'-F-CTP,

\*\* 2'-F pyrimidines,

\*\*\* 2'-OH pyrimidines,

\*\*\*\* 2'-F-UTP,2'-NH2-CTP

**Table 5**DNA oligonucleotides used in Examples 5 and 6<sup>a</sup>

Description	SEQ ID NO.
40N7 Template for RNA SELEX	TCGGGCAGTCGCTCTG[40N]CCGCATCGTCCTCCC TAATACGACTCACTATAAGGGACGGACGATGCCG
5N7 5'-primer for PCR	43
3N7 3'-primer for PCR	44
	45
40D7 Starting material for DNA SELEX	GGGAGGACGATGGGG[40N]CAGACGACTGCCGA
5D7 5'-primer for PCR	46
3D7 3'-primer for PCR	47
	48
40N8 Template for RNA SELEX	GCTGTGAGCCCTCTGTCGAA[40N]TTGAGCGTTTATTCTTGTCCTCC
5N8 5'-primer for PCR	49
3N8 3'-primer for PCR	50
	51
40D8 Starting material for DNA SELEX	GGGAGACAAGAATAACGCTCAA[40N]TTGACAGGGAGGCTCACAAACAGGC
5D8 5'-primer for PCR	52
3D8 3'-primer for PCR	53
	54

a. DNA oligonucleotides 40N7 and 40N8 were used to generate the double-stranded DNA template for *in vitro* transcription. The 3'-primers 3N7 and 3N8 were also used to generate cDNA from the RNA repertoire. Synthetically synthesized DNA oligonucleotides 40D7 and 40D8 were used directly as the starting repertoire for the two single-stranded DNA SELEX experiments. PCR amplification of the selected repertoires used the appropriate 5'- or 3'-primer. The symbol 40N indicated a 40-nucleotide randomized region within the oligonucleotide.

Table 6

**TGFB1 40N7 DNA Selex Sequence of  
fifty randomly chosen clones.**

5'    GGGAGGACGATGCGG...40N...CAGACGACTCGCCCGA    3'

		SEQ ID
		NO.
<b>Group A</b>		
20 (11 clones)	CCAGGGGGGGTATGGGGGTGGTGCTACTTACTTACGTCTT	55
4	CCAGGGGGGGTATGGGGTAGTGCTACTTACGTCTT	56
5	CCAGGGGGGGTATGGGGTAGTACTACTTACGTCTT	57
8	CCAGGGGGGGTATGGGGTAACTACTTACGTCTT	58
13	CCAGGGGGGGTATGGGGTAATACTACTTACATCTT	59
16	CCAGGGGGGGTATGGGGTAATACTACTTACGTCTT	60
40	CCAGGGGGGGTATGGGGTGGTACTTACGTCTT	61
48	CCAGGGGGGGTATGGGGTGGTCTTACTTACGTCTT	62
<b>Group B</b>		
18	CCAGGGGGGGTATGGGGTGGTACTTTCTCGTCTTC	63
19	CCAGGGGGGGTATGGGGTGGTCTTCTTGCCTTC	64
32	CCAGGGGGGGTGTGGGGTGGTACTTTCTGTCTTC	65
46	CCAGGGGGGGTATGGGGTGGTTGGTATGTTGCCTTC	66
<b>Group C</b>		
12 (3 clones)	CCGGGGTGGTATGGGGTAATACTACTTACGTCTT	67
1	CCGGGGTGGTACGGGGTAGTGCTACTTACGTCTT	68
3	CCAGGGTGGTGGTAGTGCTACTTACGTCTT	69
10	CCAGGGTGGTAGTGCTACTTACGTCTT	70
23	CCGGGGTGGTATGGGGTGGTACTTACGTCTT	71
34	CCTGGGTGGTATGGGGTGGTACTTACGTCTT	72
<b>Group D</b>		
2	CCACGGGTGGTGTGGGTAGTGTCTCACTTACATCAC	73
6	CCCCGGGTGGGTGTGGGTAGTGTATTATTTACAGCCT	74
25 & 38	CCAGGGTCGGTGTGGGTGGTACTTTCTGTCTTC	75
7	CCAGGGTCGGTATGGGTAGTGTACTTTTAATGATCTTC	76
9	CCCCGGGGAGAGCGGTGGTAGTGTCTATAGTATCGTGT	77
11	CCAGGGGGGTATGTTTAATACTACTTACGTCTT	78
17	CCAGGGAGGGTATGGGGTGGTCTAGTTGCCTTC	79
21	CCAGGGTGGCATGGGGTGGTGGATTAAATTCTCGTCC	80
24	CCAGGGTCGGTGTGGGTGGTTTATTACTCGTCC	81
28 & 30	GGGGCGGCTTGAAGAGGGTGCCGGTTGGAGTATCGAGC	82
29	CCAGGTGTGGGTGGTTGGTCTTCGTGCC	83
31	CCAGGGTGGTATGGGGTTAACCTTCGTCCCA	84
35	GGGGCGGCTTGAAGAGGGTGCCGGTTGGAGTATCGAGC	85
36	CCCCGGGTGGCTGGGGTGGTGTGAATTAAATTCTCGTCC	86
41	CCCCGGGTGGCTGGGGTGGTGTATTATATTGCGGCCT	87
44 & 45	CCAGGGTCGGTGTGGGTGGTACTTTCTGTCTTC	88
50	GGGGCGGCTTGAAGAGGGTGCCGGTTGGAGTATCGAGC	89

Bold typeface indicates a discrepancy with the most common sequence of that group.

**Table 7**  
**Starting DNA and PCR primers for the ssDNA SELEX experiment**

	SEQ ID NO.
Starting ssDNA: 5'-ATCCGCCTGATTAGCGATACT[-40N-]ACTTGAGCAAAATCACCTGCAGGGG-3'	90
PCR Primer 3N2*: 5'-BBBCCCCTGCAGGTGATTTGCTCAAGT-3'	91
PCR Primer 5N2**: 5'-CCGAAGCTTAATACGACTCACTATAGGG <u>ATCCGCCTGATTAGCGATACT</u> -3'	92

\*B=biotin phosphoramidite (e. g., Glen Research, Sterling, VA)

\*\*For rounds 10, 11, and 12, the truncated PCR primer 5N2 (underlined) was used to amplify the template.

**Table 8**  
**Unique Sequences of the ssDNA high affinity ligands to PDGF**

5'-ATCCGCCTGATTAGCGATACT [40N] ACTTGAGCAAAATCACCTGCAGGGG-3'		SEQ ID NO
*14	AGGCTTGACAAAGGGCACCATGGCTTAGTGGTCTAGT	93
*41	CAGGGCACTGCAAGCAATTGTGGTCCCAATGGGCTGAGT	94
6	CCAGGCAGTCATGGTCATTGTTACAGTCGTGGAGTAGGT	95
23	AGGTGATCCCTGCAAAGGCAGGATAACGTCTGAGCATC	96
2	ATGTGATCCCTGCAGAGGGAGGGANACGTCTGAGCATC	97
34	CACGTGATCCCATAAGGGCTGCGAAAATAGCAGAGCATC	98
8	GGTGGACTAGAGGGCAGCAAACGATCCTGGTAGCGTCC	99
1	GGTGCAGCAGGGCTTACACAAACGTACACGTTCCCCGC	100
5	TGTCGGAGCAGGGCGTACGAAAACTTACAGTTCCCCCG	101
*40	AGTGGAACAGGGCACGGAGAGTCAAACATTGGTTCCCC	102
47	GTGGGTAGGGATCGGTGGATGCCCTCGTCACTTCTAGTCCC	103
18	GGGCGCCCTAAACAAAGGGTGGTCACTTCTAGTCCCAGGA	104
30	TCCGGGCTCGGGATTCGTGGTCACTTCACTCCCAGATA	105
*20	ATGGGAGGGCGCCTTCTCGTGGTTACTTAGTCCC	106
35	ACGGGAGGGCACGTTCTCGTGGTTACTTAGTCCC	107
13	GCTCGTAGGGGGCATTCTTCGCCCTACTTCCAGTCCT	108
16	GAGGCATTTAACATGAGCATCGTCTCACGATCCTCAGCC	109
*36	CCACAGGCTACGGCACGTAGAGCATCACCATGATCCTGTG	110
50	CGGGGCATGGCACATGAGCATCTCTGATCCCGCAATCCTC	111
4	ACCGGGCTACTTCGTAGAGCATCTCTGATCCCGGTCTCG	112
44	AAAGGGCGAACGTAGGTGAGGCATCCATTGGATCCCTTC	113
24	ACGGGCTCTGTCACTGTGGCACTAGCAATAGTCCCCTGC	114
7	GGGCAGACCTTCTGGACGAGCATCACCTATGTGATCCC	115
*26	AGAGGGGAAGTAGGCTGCCTGACTCGAGAGAGTCCCTCCC	116
19	AGGGGTGCGAACACATAATCCTCGGGATTCCCATCGCT	117
48	GGGGGGCAATGGCGTACCTCTGGTCCCCTAAATAC	118
46	GCGGCTCAAAGTCTGCTACCCGAGCACATCTGTGGTC	119
25	TTGGGCGTGAATGTCACGGGTACCTCCGGTCCCAAAGAG	120
31	TCCGCGCAAGTCCCTGGTAAGGGCAGCCCTAAGTGGTC	121
12	CAAGTCCCCACAAGACTGGGGCTGTTCAAACCGCTAGTA	122
15	CAAGTAGGGCGCGACACACGTCCGGGACCTAAGGTCCA	123
*38	AAAGTCGTGCAGGGTCCCCTGGAAGCATCTCCGATCCCAG	124

\* Indicates a boundary experiment was performed.

Italics indicate the clones that were found to retain high affinity binding as minimal ligands.

Table 9

**Table 10**  
**Frequency of base pairs in the helical regions of the consensus motif**  
**shown in Figure 3**

<u>Position<sup>a</sup></u>	Base pair <sup>b</sup>						
	<u>AT</u>	<u>TA</u>	<u>GC</u>	<u>CG</u>	<u>TG</u>	<u>GT</u>	<u>other</u>
I-1	0	0	21	0	0	0	0
I-2	0	0	21	0	0	0	0
I-3	5	0	16	0	0	0	0
I-4	3	5	1	4	1	0	7
I-5	2	3	3	4	0	0	9
II-1	0	1	2	17	0	0	1
II-2	5	5	5	1	0	4	1
II-3	3	4	7	6	0	0	1
II-4	3	0	8	5	0	0	4
III-1	21	0	0	0	0	0	0
III-2	0	10	0	11	0	0	0
III-3	0	7	0	13	1	0	0

<sup>a</sup>Helices are numbered with roman numerals as shown in Figure 3. Individual base pairs are numbered with arabic numerals starting with position 1 at the helix junction and increasing with increased distance from the junction.

<sup>b</sup>We have included the TG and GT base pairs to the Watson-Crick base pairs for this analysis. There is a total of 21 sequences in the set.

**Table 11**  
**Affinities of the minimal DNA ligands to**  
**PDGF AA, PDGF AB and PDGF BB**

<u>Ligand</u>	$K_d$ , nM		
	<u>PDGF AA<sup>a</sup></u>	<u>PDGF AB<sup>b</sup></u>	<u>PDGF BB<sup>b</sup></u>
20t	47 ± 4	0.147 ± 0.011	0.127 ± 0.031
36t	72 ± 12	0.094 ± 0.011	0.093 ± 0.009
41t	49 ± 8	0.138 ± 0.009	0.129 ± 0.011

<sup>a</sup>Data points shown in Figure 5A were fitted to eq 1 (Example 7).

<sup>b</sup>Data points in Figures 5B and 5C were fitted to eq. 2. The dissociation constant ( $K_d$ ) values shown are for the higher affinity binding component. The mole fraction of DNA that binds to PDGF AB or PDGF BB as the high affinity component ranges between 0.58 to 0.88. The  $K_d$  values for the lower affinity interaction range between 13 to 78 nM.

**Table 12**  
**Starting RNA and PCR primers for the 2'-fluoropyrimidine**  
**RNA SELEX experiment**

	SEQ ID NO
Starting 2'-fluoropyrimidine RNA:	
Starting RNA:	
5'-GGGAGACAAGAAUAACGUCAA[ -50 N -] UUCGACAGGAGGCUCACAAACAGGC-3'	125
PCR Primer 1:	
5'-TAATACGACTCACTATAGGGAGACAAGAATAACGCTCAA-3'	126
PCR Primer 2:	
5'-GCCTGTTGTGAGCCTCCTGTCGAA-3'	127

**Table 13**  
**Sequences of the 2'-fluoropyrimidine RNA high affinity ligands to PDGF AB.**

	SEQ ID NO.
1	128
2	129
3	130
4	131
5	132
6	133
7	134
8	135
10	136
13	137
15	138
16	139
17	140
18	141
19	142
20	143
21	144
22	145
23	146
24	147
26	148
27	149
29	150
30	151
31	152
32	153
33	154
34	155
35	156
39	157
41	158
42	159
43	160
44	161
45	162
46	163
47	164
49	165
51	166
52	167
53	168
54	169
55	170

**Table 14****Starting RNAs:**

40N7:

5'GGGAGGACGAUGC GG[-40N-]CAGACGACUCGCCGA 3' (SEQ ID NO: 186)

**SELEX PCR Primers:**

5G7:

5'TAATACGACTCACTATAGGGAGGACGATGCGG 3' (SEQ ID NO: 187)  
T7 Promoter

3G7:

5'TCGGGCGAGTCGTCTG 3' (SEQ ID NO: 188)

**Table 15**  
**Conditions and progress of the SELEX against hKGF**

Round	[RNA], M	[KGFL], M	net % bound	Signal/noise	PF*	Spin <sup>b</sup>	B-Wash <sup>c</sup> (ml)	U-Wash <sup>d</sup> (ml)	SPKD <sub>r</sub> , M	KD <sub>s</sub> , nM
1	1.00E-06	3.00E-07	4.4	11.8	4				5.61E-06	30.0
2	4.00E-06	3.00E-07	1.5	4.2					1.58E-05	
3	1.00E-06	1.00E-07	5.9	20.6					8.52E-07	
4	1.00E-06	1.00E-07	14.3	12.8	+				3.21E-06	17.0
5	3.00E-07	1.00E-08	2.5	4.5	+				7.64E-08	
6	3.70E-08	3.70E-09	0.7	2.6	+	+	15	15	3.73E-07	
7	4.10E-09	4.10E-10	1.1	8.2	+	+	20	20	2.46E-08	0.7
8	4.60E-10	4.60E-11	1.5	8.8	+	+	25	25	2.04E-09	0.3
9	5.10E-11	5.10E-12	0.7	5.9	+	+	25	25	8.76E-10	
10	1.70E-11	1.70E-12	0.3	2.1	+	+	25	25	4.12E-10	
<b>2F SELEX</b>										
1	1.00E-06	3.00E-07	2.9	11.0				4	3.39E-06	
2	4.00E-06	3.00E-07	2.2	9.9				5	9.28E-06	
3	3.00E-06	3.00E-07	5.7	5.7				5	2.15E-06	
4	2.50E-06	3.00E-07	3.9	11.7	+			8	4.98E-06	15.0
5	6.70E-07	3.00E-08	2.3	5.8	+			8	3.64E-06	
6	1.20E-08	1.23E-09	0.3	1.8	+			15	1.59E-07	
7	1.40E-09	1.40E-10	1.1	11.2	+	+		20	6.86E-09	0.6
8	1.50E-10	1.50E-11	0.4	4.8	+	+		25	5.36E-10	0.3
9	1.70E-11	1.70E-12	0.2	3.1	+	+		25	5.67E-10	
10	1.70E-11	1.70E-12	0.3	3.0	+	+		25	1.42E-10	

\*Prefiltered RNA through nitrocellulose to counter select for nitrocellulose binding molecules

<sup>b</sup>Brief spinning of the binding reactions

<sup>c</sup>Volume of buffer used to wash the captured complexes

<sup>d</sup>Volume of 0.5M urea wash following the buffer wash

<sup>e</sup>Calculated single point K<sub>d</sub> from the binding data at each round  
<sup>f</sup>K<sub>d</sub> values obtained from binding curves

Clone	S constant	random
<u>2'-NH<sub>2</sub> ligands:</u>		
IN	GCTGAGGACCAUUGCCG GAAUCCGAAUAGCAGAAUCCGAACAAAGGCGGUCGGC	
2N	GGGAGGACCAUUGCCG GGGGGAAUCCGAAUACCCGAAAGGAAACGAGAUUCC	
4N	GGGAGGACCAUUGCC GUGGUGAAGGUACCCGAUUCGUUAAGAUACCACGCC	
6N	GGGAGGACCAUUGCC GGAGGGACCAUUGACUCAUGCAAGCCGUGGCACGUGG	
10N	GGGAGGACCAUUGCC GAGCAGGACCAUUGACUCAUGCAAGACACUAGAACGUUAGUUGGG	
11N	GGGAGGACCAUUGCC GGGCCGAAUUGAACCGGACUUCGGGUUAGGCCCCGACGU	
14N	GGGAGGACCAUUGCC UCCAGGGAUUGAAGGUUCGGGUAGGAACAAAGCCGC	
16N	GGGAGGACCAUUGCC AAGIUCUUAACAGUUAUUGGAAGGUUCCACUUGAUUGUA	
22N	GGGAGGACCAUUGCC AUGCAGCUGAAAU	
24N	GGGAGGACCAUUGCC GUGGGAAAGAUUAGCCGAGUAAUUGACACUAGGCGG	
25N	GGGAGGACCAUUGCC GATGGGAAGUAGGAAACACUACUAGCAGAUAAACGGG	
27N	GGGAGGACCAUUGCC AUUGAGCUGAAAU	
28N	GGGAGGACCAUUGCC UUCCUCHUACAUAGACGGGHIIGACUCCGGAGIUCUUGACA	
29N	GGGAGGACCAUUGCC GAGGGGAGGAAGGAUUCAGGAACAGCGAAAUUGCGUHGGC	
34N	GGGAGGACCAUUGCC GCGGGAAAGGCGUAAUUGGAAGGGAAUACAGUUCACAGGCGG	
35N	GGGAGGACCAUUGCC GCUUAGGGAAAUUGGUUCUGAGGUUGGU	
36N	GGGAGGACCAUUGCC GAAGGGAAACAGGAUAGACAAGUGGAACAAAGCCGAGGU	
37N	GGGAGGACCAUUGCC AUUGAGCUGAAAU	
42N	GGGAGGACCAUUGCC GGAGACGUAGACGGGAACAUAGGAACAGCAUACGCGGC	
43N	GGGAGGACCAUUGCC GIAAGUGGUUAAGAACAGUCAAGAACGGGUAGGUG	
47N	GGGAGGACCAUUGCC GAAGGGGUAGGAAGGGCAAGGGAAACGCGCUUCGGGUG	
48N	GGGAGGACCAUUGCC GCGAAAGGAAGUUGGAUUCGGACUAAUUGUAGGUHGGC	
54N	GGGAGGACCAUUGCC AGAACCAAAGGCCCCTGGGGGGGGAGGGAAUUCGGGUAGG	
55N	GGGAGGACCAUUGCC ACACACAAUAGGAAGGUACAGCCGAUUIACGGGGGG	
57N	GGGAGGACCAUUGCC UCCUGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	
59N	GGGAGGACCAUUGCC GGUGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	
60N	GGGAGGACCAUUGCC CCCCUUAGCUCAGGGGUAGAG	
65N	GGGAGGACCAUUGCC UAACGGGAAUAGGGGUAAAACGGGGAAUAAACGGGG	
69N	GGGAGGACCAUUGCC GAGGGAGGUACAGUACGGGGGGGGGGGGGGGGGGGGGG	

Table 16 (Page 2)

Clone	5' constant	random	3' constant	SEQ ID NO.
71N	GGGAGGAAGCGAUGGGGAAACGGCGUGGCCAAGUAAAAGUGAGGGGUAGGAUGGUACCAUGGU		CAGACGACUCCGGCA	218
72N	GGGAGGAAGCGAUGGGGAGGGGAAAGUAGGACAGATTCACGGAAUAGUACUGGG		CAGACGACUCCGGCA	219
2F	GGGAGGAAGCGAUGGGGCAUUCGGUAAUACCUUUGGUUUUAUCCUUUUCUAGCGGCC		CAGACGACUCCGGCA	220
3F	GGGAGGAAGCGAUGGGGAUCGUAAUCCCAACUACAUUCCGUACCCGACAGGUGGC		CAGACGACUCCGGCA	221
5F	GGGAGGAAGCGAUGGGCGUCCCGAGGACUGCGUCCGUAGAAACGUUUCUUGGGC		CAGACGACUCCGGCA	222
6F	GGGAGGAAGCGAUGGGGAUCGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	223
7F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	224
8F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	225
9F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	226
10F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	227
11F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	228
12F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	229
13F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	230
14F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	231
15F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	232
16F	GGGAGGAAGCGGCGGGGUACGUUUCGGGUACGGCAGGTCCGGUAGGUUUCGGGCC		CAGACGACUCCGGCA	233
19F	GGGAGGAAGCGGCGGGCAA-NNNNNNNCUUCUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	234
20F	GGGAGGAAGCGGCGGGAGUAUCCAGCGTGUAGGUUACGUUAGGUUACGUUAGGUUCC		CAGACGACUCCGGCA	235
21F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	236
22F	GGGAGGAAGCGGCGGGCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	237
23F	GGGAGGAAGCGGCGGGCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	238
24F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	239
26F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	240
27F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	241
28F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	242
29F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	243
31F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	244
35F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	245
37F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	246
38F	GGGAGGAAGCGGCGGGUCCUAGCGTAUCAUCCGUUCCGUUCCGUUCCGUUCCGUUCC		CAGACGACUCCGGCA	247

Table 16 (Page 3)

Clone	5' constant	random	3' constant	SEQ ID NO.
41F	GGGAGGACCAUAGCGG GGACUUUGACAGGC AUUUGACCU UGUAUUCGCCGUUCCCCGUAGC	CAGACGACUCCGCCGA		248
42F	GGGAGGACCAUAGCGG CGACACAUAAGCCAUUUGAUAUCCAUUAGGCUUCCGUUCCCCGUAGC	CAGACGACUCCGCCGA		249
43F	GGGAGGACCAUAGCGG UGUAUHUUCCUGUAUCCAUUAGC AUUCCAUUCCGUUCCCCGUAGC	CAGACGACUCCGCCGA		250
44F	GGGAGGACCAUAGCGG UCGAGUHGUUCCUUCGUUCCCCGUAGC AUUINNNNAUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		251
45F	GGGAGGACCAUAGCGG GUCCUAUUCGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		252
49F	GGGAGGACCAUAGCGG GGACUUUGACAGGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		253
50F	GGGAGGACCAUAGCGG UGAUCAUCCUUCGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		254
51F	GGGAGGACCAUAGCGG CAGUCCUCCUAGGUUUAUCCUUCGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		255
53F	GGGAGGACCAUAGCGG AUCAAAAGGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		256
54F	GGGAGGACCAUAGCGG AAGAUUCCUACGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		257
55F	GGGAGGACCAUAGCGG UCCGUUUAACGGCAUAAACUGGAAAUACUCCUCCUGGCC	CAGACGACUCCGCCGA		258
56F	GGGAGGACCAUAGCGG GGACAAWYAGGGGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		259
57F	GGGAGGACCAUAGCGG UGACUAUUCUCCGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		260
58F	GGGAGGACCAUAGCGG UGACUAUUCUCCGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		261
60F	GGGAGGACCAUAGCGG UGACUAUUCUCCGUUCCCCGUAGC AUUUCGUUCCCCGUAGC	CAGACGACUCCGCCGA		262

**Table 17**  
 **$K_d$  values hKGF ligands**

Clone	$K_d$ in nM		Clone	$K_d$ in nM	
	1	2		1	2
1N	0.51		2F	1.77	
2N	0.77		3F	4.47	
4N	0.75		5F	2.53	
6N	0.71		6F	0.05 (37)	3.25
10N	1.10		7F	3.69	
11N	1.28		8F	2.63	
14N	0.44		9F	0.83	
16N	1.40		10F	0.47	
22N	5.70		11F	3.74	
24N	1.16		12F	1.38	
25N	0.87		13F	0.03 (28)	
27N	ND		14F	0.006-0.03 (25-44)	
28N	2.54		15F	0.07 (33)	
29N	0.43		16F	0.83 (49)	3.39
34N	0.80		19F	1.6	0.94-2.57
35N	2.32		20F	2.05	8.70
36N	8.27		21F	ND	44.8
37N	ND		22F	2.75	
42N	0.78		23F	2.52	
43N	0.79		24F	2.02	
47N	1.76		26F	0.23 (43)	2.55
48N	1.34		27F	1.52	
54N	5.35		28F	ND	
55N	1.25		29F	3.24	
57N	35.8		31F	1.0	
59N	22.0		35F	1.1	
60N	7.38		37F	0.46	
65N	26.56		38F	0.33	
69N	15.20		41F	1.44	
71N	3.52		42F	0.9	
72N	7.67		43F	1.13	
random	30		44F	1.32	
			45F	4.7	
			49F	1.0	
			50F	0.12 (21)	2.10
			51F	1.27	
			53F	0.70	
			54F	1.23	
			55F	2.52	
			56F	0.07 (32)	3.00
			57F	1.20	
			58F	2.52	
			60F	2.10	
			random	30	

For biphasic curves,  $K_d1$  is for the high affinity component.

Number in parentheses indicate the per cent of the high affinity component.

100

**Table 18****Binding Specificity of the 2'-F Ligand K14F**

Target	Ratio:
	$K_D$ Target/ $K_{Dh}$ KGF
human hKGF	1
rat hKGF	1,254
human aFGF	38,650
human bFGF	1,071
human PDGF	432

The ratios shown are averages of at least two determinations

**Table 19****IC<sub>50</sub> values from the PC-3 assay**

Competitor	IC <sub>50</sub> , nM
hKGF	70
Heparin, 5,000	30
40N7F	>1000
K6F	4
K13F	30
K14F	10
K15F	20
K56F	1
K10F	30
K37F	20
K38F	0.6
K43F	80
40N7N	>1000
K1N	50
K2N	200
K4N	70
K6N	80
K14N	6
K29N	40
K42N	800
K43N	800

Table 20

**K<sub>i</sub> values of hKGF competitors on the PC3 and NIH3T3/FGFR-2 competition assay**

<u>Cell line</u>	<u>Competitor</u>	<u>K<sub>i</sub>, nM</u>	<u>R</u>
PC-3	hKGF	7.700	0.95519
	2'F random	930.000	0.99713
	2'NH <sub>2</sub> random	673.000	0.85357
	Hep5000	6.500	0.99984
	K14F	0.200	0.97735
	K6F	0.160	0.95927
	K38F	0.220	0.99013
	K56F	0.160	0.95927
NIH3T3/FGFR-2	hKGF	0.034	0.9933
	2'F random	>10,000.000	
	2'NH <sub>2</sub> random	>10,000.000	
	Hep5000	26.300	0.97856 partial comp.
	K14F	2.700	0.99047
	K6F	6.800	0.96202
	K38F	20.000	0.98659
	K56F	27.400	0.97582
	K14N	10.600	0.97856 partial comp.

Table 21

**I<sub>C50</sub> values obtained with the gel shift assay**

<u>Competitor</u>	<u>I<sub>C50</sub>, nM</u>
KGF	70
bFGF	1,500
Lysozyme	10,000

Table 22

## Binding Specificity of Ligand K14F3'T

Protein	random RNA		K14F3'T		DF
	<sup>a</sup> $K_d$ 1, nM	<sup>b</sup> $K_d$ 2, nM	$K_d$ 1, nM	$K_d$ 2, nM	
hKGF		20.1	0.0008	10.2	1
rKGF		45.3	0.0041	70.0	5
hbFGF	0.0375	10.3		10.0	$1.2 \times 10^4$
haFGF		16,000,000		24,000,000	$3 \times 10^{10}$
hPDGF-AB		22.0		50.0	$6.2 \times 10^4$
hTGF $\beta$ 1		10.4		98.0	$1.2 \times 10^5$
hEGF		2,000		256	$3.2 \times 10^5$
Thrombin		7,200,000		22,700,000	$2.8 \times 10^{10}$

<sup>a</sup>High affinity dissociation constant from biphasic binding curves.<sup>b</sup>Low affinity dissociation constant from biphasic binding curves or affinity dissociation constant from monophasic binding curves.Discrimination factor defined as the ratio of the highest affinity  $K_d$  of 14F3'T for the corresponding protein over the affinity  $K_d$  for hKGF.

Table 23

		SEQ ID NO.: 272	S1 GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   <u>CCAUCGUAAUC</u> L1 GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC S2' GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC L3 GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC S2 GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC H GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC L GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC
T2	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC .. .	-	273
T3	GGGAGG   AC   .. UGGGGUCC   UCUCCC   AAUUCUAACUUUCU   CCAUCGUAAUC .. .	-	274
T4	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   .. . CUAACUUUCU   CCAUCGUAAUC .. .	- +	275
T5	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . UUCU   CCAUCGUAAUC .. .	+ +	276
T6	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACU .. .	+ +	277
T7	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACU .. .	+ -	278
T8	.. . GAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACU .. .	+ -	279
T9	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUAACU .. .	-	280
T10	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	281
T11	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	282
T12	.. . GGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	283
T13	. GGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	284
T14	.. . GAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	285
T15	.. . GAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	286
T16	GGGAGG   . C   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	287
T17	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	288
T18	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	289
T19	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	290
T20	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	291
T21	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	292
T22	GGGAGG   AC   <u>GAUGGGGUCC</u>   UCUCCC   AAUUCUA .. . . . .	-	293
T23	GGG. G.   AC   <u>GAUGGGGUCC</u>   . CCC   AAUUCUA .. . . . .	-	294
T24	GGG. .   AC   <u>GAUGGGGUCC</u>   . CCC   AAUUCUA .. . . . .	-	295
T25	.. . GG   AC   <u>GAUGGGGUCC</u>   . CCC   AAUUCUA .. . . . .	-	

SREQ ID  
NO:

Table 23 (Page 2)

	S1	L1	S2	S1'	I.3	S2'	H	L
T33	...	...	GAUGCGGGG   . . . CCC   AAUUCUA . . . UUCU   CCAUCCGUAC				296	
T34	...	...	GAUGCGGGG   UCUCCC   AAUUCUA . . . UUCU   CCAUCCGUAC				297	
T35	...	...	. . . GUGGG   UCUCCC   AAUUCUA . . . UUCU   CCAUCCGUAC				298	
T36	GGGAGG	AC	GAUG . . . . .   . . . . .   . . . . .   . . . . .   . . . . .   . . . . .   . . . . .   . . . . .   . . . . .				299	
T37	GGGAGG	AC	GAUGCGGGG   UCUCCC   AAUUC . . . . .   UCU   CCAUCCGUAC				300	
T39	GGGAGG	AC	GAUGCGGGG   UCUCCC   AAUUC . . . . .   UUCU   . CAUCCGUAC				301	
T40	GGGAGG	AC	GAUGCGG . GG   UCUCCC   AAUUC . . . . .   UUCU   CC . UCCGUAC				302	
T41	GGGAGG	AC	GAUGCGGG . G   UCUCCC   AAUUC . . . . .   UUCU   C . UCCGUAC				303	
T22mu	GGGAGU	AC	GAUGCGGGG   UCUCCC   AAUUC . . . . .   UUCU   CCAUCCGUAC				304	
T35 / 36			equimolar amounts of T35 and T36					

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: LARRY GOLD; NEBOJSA JANJIC; STEVEN RINGQUIST;  
NIKOS PAGRATIS; PENELOPE J. TOOTHMAN  
(ii) TITLE OF THE INVENTION: HIGH AFFINITY OLIGONUCLEOTIDE  
LIGANDS TO TRANSFORMING GROWTH  
FACTOR  $\beta$  (TGF $\beta$ ), PLATELET-DERIVED  
GROWTH FACTOR (PDGF) AND HUMAN  
KERATINOCTYE GROWTH FACTOR (hKGF)

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 Mb storage  
(B) COMPUTER: IBM compatible  
(C) OPERATING SYSTEM: MS-DOS  
(D) SOFTWARE: WordPerfect 6.1

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US96/  
(B) FILING DATE:  
(C) CLASSIFICATION:

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/458,423  
(B) FILING DATE: 02-JUNE-1995

## (viii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/458,424  
(B) FILING DATE: 02-JUNE-1995

## (ix) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/465,594  
(B) FILING DATE: 05-JUNE-1995

## (x) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/465,591  
(B) FILING DATE: 05-JUNE-1995

## (xi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/479,725  
(B) FILING DATE: 07-JUNE-1995

## (xii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/479,783  
(B) FILING DATE: 07-JUNE-1995

## (xiii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/618,693  
(B) FILING DATE: 20-MARCH-1996

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- (2) INFORMATION FOR SEQUENCE ID NO: 1:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:  
 GGGGGAGAAC GCGGAUCCNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  
 NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNAAGCUUCG CUCUAGAUCA      50  
 CCCUUUAGUG AGGGUUA      100  
 CCCUUUAGUG AGGGUUA      117
- (2) INFORMATION FOR SEQUENCE ID NO: 2:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 93 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:  
 GGGGGAGAAC GCGGAUCCNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  
 NNNNNNNNAA GCUUCGCUCU AGAUCUCCCU UUAGUGAGGG UUA      50  
 NNNNNNNNAA GCUUCGCUCU AGAUCUCCCU UUAGUGAGGG UUA      93
- (2) INFORMATION FOR SEQUENCE ID NO: 3:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 123 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:  
 GGGGGAGAAC GCGGAUCCUG UCUCACCACGC CGAUACUGGG GUUCCUGGGG  
 CCCCCUCCAUG GAGGAGGGGG GAGGGGGUGG UUCGGAGAAA GCUUCGCUCU      50  
 GAAUCUCCCU UUAGUGAGGG UUA      100  
 GAAUCUCCCU UUAGUGAGGG UUA      123
- (2) INFORMATION FOR SEQUENCE ID NO: 4:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 95 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:  
 GGGAGAACGC GGATCCNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  
 NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN AAGCTTCGCT CTAGA      50  
 NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN AAGCTTCGCT CTAGA      95
- (2) INFORMATION FOR SEQUENCE ID NO: 5:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:  
GGGAGAACGC GGATCCNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  
NNNNNNNAAGC TTCGCTCTAG A 50  
71

(2) INFORMATION FOR SEQUENCE ID NO: 6:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 95 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:  
GGGGGAGAAC GCGGATCCTG TCTCCACCGC CGATACTGGG GTTCCTGGGG  
CCCCCTCCATG GAGGAGGGGG TGTTCTGGAG AAAGCTTCGC TCTAG 50  
95

(2) INFORMATION FOR SEQUENCE ID NO: 7:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 35 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:  
TAATACGACT CACTATAGGG GGAGTCTGCG GATCC 35

(2) INFORMATION FOR SEQUENCE ID NO: 8:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 35 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:  
TAATACGACT CACTATAGGG GGAGAACGCG GATCC 35

(2) INFORMATION FOR SEQUENCE ID NO: 9:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 35 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:  
TAACCCTCAC TAAAGGGAGA TCTAGAGCGA AGCTT 35

(2) INFORMATION FOR SEQUENCE ID NO: 10:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 50 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GATTTAGGTG ACACTATAGA ATATGCATCA CTAGTAAGCT TTGCTCTAGA 50

(2) INFORMATION FOR SEQUENCE ID NO: 11:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGATCCCGGA GCTCCCTATA GTGAGTCGTA TTA 33

(2) INFORMATION FOR SEQUENCE ID NO: 12:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 125 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F)  
modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGGGGAGAAC GCGGAUCCGA GCAAUCCCAG GCGCAUAGCU UCCGAGUAGA 50

CAGGAGGGAG GGGUGGAUGU GGCGUCUACU CGGUGUCGUG AAGCUUCGCU 100

CUAGAUCUCC CUUUAGUGAG GGUUA 125

(2) INFORMATION FOR SEQUENCE ID NO: 13:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 125 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F)  
modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGGGGAGAAC GCGGAUCCGA GCAAACCCAG GCGCAUAGCU UCCGAGUAGA 50

CAGGAGGGAG GGGUGGAUGU GGCGUCUACU CGGUGUCGUG AAGCUUCGCU 100

CUAGAUCUCC CUUUAGUGAG GGUUA 125

(2) INFORMATION FOR SEQUENCE ID NO: 14:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 125 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F)  
modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGGGGAGAAC GCGGAUCCGA GCAAACCCAG GCGCAUAGCU UCCGAGUAGA 50

CAGGCAGGAG GGGUGGAUGU GGCGUCUACU CGGAGUCGUG AAGCUUCGU	100
CUAGAUCUCC CUUUAGUGAG GGUUA	125

## (2) INFORMATION FOR SEQUENCE ID NO: 15:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-amino (2'NH<sub>2</sub>) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GGGGGAGAAC GCGGAUCCGG CAACCCCAGG CGCAUAGCUU CCGAGUAGAC	50
AGGCAGGGAGG GGUGGAUGUG GCGUCACGAG GAAGCUUCGC UCUAGAUCUC	100
CCUUUAGUGA GGGUUA	116

## (2) INFORMATION FOR SEQUENCE ID NO: 16:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 123 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGGGGAGAAC GCGGATCCGC AAUCCCAGGC GCAUAGCUUC CGAGUAGACA	50
GGAGGGAGGG GUGGAUGUGG CGUCUACUCG GCGUCGUGAA GCUUUCGCUCU	100
AGAUCUCCU UUAGUGAGGG UUJA	123

## (2) INFORMATION FOR SEQUENCE ID NO: 17:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-amino (2'NH<sub>2</sub>) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGGGGAGAAC GCGGAUCCGA GCAAUCCAG GCGCAUAGCU UCCGAGUAGA	50
CAGGAGGGAG GGGUGGAUGU GGCGUCUCGA GGAAGCUUCG CUCUAGAUCU	100
CCCUUJAGUG AGGGUUA	117

## (2) INFORMATION FOR SEQUENCE ID NO: 18:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 123 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

110

(D) OTHER INFORMATION: All pyrimidines are 2'-amino (2'NH<sub>2</sub>) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGGGGAGAAC GCGGAUCCGA GCAAGCCCUG GCAUAGCUUC CGAGUAGACA	50
GGAGGGAGGG GUGGAUGUGG CGUCUACUCG GUGUCGUGAA GCUUCGCUCU	100
AGAUCUCCCU UUAGUGAGGG UUA	123

(2) INFORMATION FOR SEQUENCE ID NO: 19:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 115 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-amino (2'NH<sub>2</sub>) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGGGGAGAAC GCGGAUCCGG CAAUCCAGG CGCAUAGCUU CCGAGUAGAC	50
AGGAGGGAGG GGUGGAUGUG GUGUACGAGG AAGCUUCGCU CUAGAUCUCC	100
CUUUAGUGAG GGUUA	115

(2) INFORMATION FOR SEQUENCE ID NO: 20:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GGGGGAGAAC GCGGAUCCGA GCAAUCCAG GCGCAUAGCU UCCGAGUAGA	50
CAGGAGGGAG GGGUGGAUGU GGUGUCUCGA GAAGCUUCGC UCUAGAUCUC	100
CCUUUAGUGA GGGUUA	116

(2) INFORMATION FOR SEQUENCE ID NO: 21:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GGGGGAGAAC GCGGAUCCAA GCUUCGAGUA GACAGGAGGG AGGGGUGGAU	50
GUGGAGUCUC GAGAAGCUUC GCUCUAGAUC UCCCCUUUAGU GAGGGUUA	98

(2) INFORMATION FOR SEQUENCE ID NO: 22:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 113 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

111

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GGGGGAGAAC GCGGAUCCGA GCAAUCCUAA GCAUAGCUUC GAGUAGACAG	50
GAGGGAGGGG UGGAUGUGGC GUCUCGAGAA GCUUCGCUU AGAUCUCCU	100
UUAGUGAGGG UUA	113

(2) INFORMATION FOR SEQUENCE ID NO: 23:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All U's are 2'-F uracil

(ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'NH<sub>2</sub> cytosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GGGGGAGAAC GCGGAUCCGA GCAAUCCCGG GCGCAUAGCU UCCGAGGAGA	50
CAGGCAGGGAG GGGUGGAAUGU GGCGUCUCGA GAAGCUUCGC UCUAGAUUC	100
CCUUUAGUGA GGGUUA	116

(2) INFORMATION FOR SEQUENCE ID NO: 24:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All U's are 2'-F uracil

(ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'NH<sub>2</sub> cytosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGGGGAGAAC GCGGAUCCGA GCAAUCCAG GCGCAUAGCU UCCGAGUAGA	50
CAGGCAGGGAG GGGUGGAAUGU GGCGUCUCGA GAAGCUUCGC UCUAGAUUC	100
CCUUUAGUGA GGGUUA	116

(2) INFORMATION FOR SEQUENCE ID NO: 25:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GCUCUCCGAGU AGACAGGAGG GAGGGGUGGA UGUGGCGUCU AC	42
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112

## (2) INFORMATION FOR SEQUENCE ID NO: 26:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 43 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CUUCCGAGUA GACAGGAGGG AGGGGUGGAU GUGGCGUCUA CUC

43

## (2) INFORMATION FOR SEQUENCE ID NO: 27:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 78 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-amino (2'NH<sub>2</sub>) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GGGGGAGAAC CGCGAUCCGG CAACCCCCAGG CGCAUAGCUU CCGAGUAGAC  
AGGCAGGGAGG GGUGGAUGUG GCGUCACG

50

78

## (2) INFORMATION FOR SEQUENCE ID NO: 28:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-amino (2'NH<sub>2</sub>) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GGGGGAGAAC CGCGAUCCUG AGAAGGACGU CGGGGUCAAC GGGGUGAGGU  
GCAGCAGAAA GGGCCGGCAC CACAUGACGU AAAAGCUUCG CUCUAGAUCU  
CCCUUUAGUG AGGGUUA

50

100

117

## (2) INFORMATION FOR SEQUENCE ID NO: 29:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 108 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GGGGGAGAAC CGCGAUCCUG AGAAGGACGU CGGGGUGAGG UGCAGCAGAA  
AGGGCCGGCA CCACAUGACG UAAAAGCUUC GCUCUAGAUC UCCCUUUAGU  
GAGGGUUA

50

100

108

113

## (2) INFORMATION FOR SEQUENCE ID NO: 30:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 92 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GGGGGAGAAC GCGGAUCCGG UGGGAAAGUC GGAUUAUGUG UGUAGAUUUG	50
UGUGCGAAAG CUUCGCUCUA GAUCUCCCCU UAGUGAGGGU UA	92

## (2) INFORMATION FOR SEQUENCE ID NO: 31:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-amino (2'NH<sub>2</sub>) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GGAGAACGCG GAUCCUGAGA AGGACGUCGG GGUCAACGGG GUGAGGUGCA	50
GCAGAAAGGG CCGGCACCA	69

## (2) INFORMATION FOR SEQUENCE ID NO: 32:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

GGGGGAGAAC GCGGAUCCUG CUAGACCGAG GAUGCAAAGG GACAUGCAUU	50
AGGGAAACCU AUGUUAAGA ACGCGGUCGC AGAACGUUCG CUCUAGAUU	100
CCCUUUJAGUG AGGGUUA	117

## (2) INFORMATION FOR SEQUENCE ID NO: 33:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

114

GGGGGAGAAC GCGGAUCCUG CUAGACCGAG GAUGCAAAGG GACAUGCAUU	50
AGGGAAACCU AUGUAUAAGA ACGCGGUCGC AGA	83

## (2) INFORMATION FOR SEQUENCE ID NO: 34:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GGGGGAGAAC GCGGAUCCUG CUAGACCGAG GAUGCAAAGG GACAUGCAUU	50
AGGGAAACCU AUJUAUAAGA CGCGGUCGC GAAGCUUCGC UCUAGAUCU	100
CCUUUAGUGA GGGUUA	116

## (2) INFORMATION FOR SEQUENCE ID NO: 35:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All C's are 2'-F uracil

## (ix) FEATURE:

- (D) OTHER INFORMATION: All U's are 2'NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GGGGGAGAAC GCGGAUCCUG UCUCCACCGC CGAUACUGGG GUUCCUGGGG	50
CCCCUCCAUG CAGGAGGGGG GUGGUUCGGGA GAAAGCUUCG CUCUAGAUCU	100
CCCUUUAGUG AGGGUUA	117

## (2) INFORMATION FOR SEQUENCE ID NO: 36:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All C's are 2'-F uracil

## (ix) FEATURE:

- (D) OTHER INFORMATION: All U's are 2'NH<sub>2</sub> cytosine

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GGGGGAGAAC GCGGAUCCUG UCUCCACCGC CGAUACUGGG GUUCCUGGGG	50
CCCCUCCAUG CAGGAGGGGG GUGGUUCGGGA G	81

## (2) INFORMATION FOR SEQUENCE ID NO: 37:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
   (D) OTHER INFORMATION: All C's are 2'-F uracil  
 (ix) FEATURE:  
   (D) OTHER INFORMATION: All U's are 2'NH<sub>2</sub> cytosine  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:  
 GGGGGAGAAC GCGGAUCCUG UCUCACCACG CGAUACUGGG GUUCCUGGGG       50  
 CCGCUCCAUG CAGGAGGGGG GUGGUUCGGA GAAAGCUUCG CUCUAGAUCU       100  
 CCCUUUAGUG AGGGUUA   117

(2) INFORMATION FOR SEQUENCE ID NO: 38:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 115 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
   (D) OTHER INFORMATION: All C's are 2'-F uracil  
 (ix) FEATURE:  
   (D) OTHER INFORMATION: All U's are 2'NH<sub>2</sub> cytosine  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:  
 GGGGGAGAAC GCGGAUCCUG UCUCACCACG CGAUACUGGG GUUCCUGGGG       50  
 CCCCUCUCCAUG CAGGAGGGGU GGUUCGGAGA AAGCUUCGCU CUAGAUCUCC   100  
 CUUUAGUGAG GGUUA   115

(2) INFORMATION FOR SEQUENCE ID NO: 39:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 92 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
   (D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F)  
     modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:  
 GGGGGAGAAC GCGGAUCCGG AAGUCUGGGUC UUUGGGGAGU CCGCAUGGCC       50  
 CUGGCGAAAG CUUCGCUCUA GAUCUCCUU UAGUGAGGGU UA               92

(2) INFORMATION FOR SEQUENCE ID NO: 40:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 111 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
   (D) OTHER INFORMATION: All pyrimidines are 2'Fluoro (2'-F)  
     modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:  
 GGGGGAGAAC GCGGAUCCAA GAAUGUUCGG CCGCACGAGG UGACAGUGGU       50  
 GCGGAUACGG ACCGAUUGGG UUUGCCAAGC UUCGCUCUAG AUCUCCCCUUU   100

AGUGAGGGUU A

111

## (2) INFORMATION FOR SEQUENCE ID NO: 41:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GGGGGAGAAC GCGGAUCCGG UCACCCGGGC AUAAUAACAAU GCCGACACUG	50
GGGUACCUGG GACGGGUGGG ACUGGGACCGGA AGAAGCUUCG CUCUAGAU	100
CCCUUUAGUG AGGGUUA	117

## (2) INFORMATION FOR SEQUENCE ID NO: 42:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 119 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

GGGGGAGAAC GCGGAUCCAU AACCGGCUGC AUGGGAGGGGA CAUCCUGGG	50
AAGGACGGGU CGAGAUGACC UGAGCAGUUC CGGCAAGCUU CGCUCUAGAU	100
CUCCCCUUUAG UGAGGGUUA	119

## (2) INFORMATION FOR SEQUENCE ID NO: 43:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

TCGGGGCAGT CGTCTGNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN	50
NNNNNNCCGC ATCGTCCTCC C	71

## (2) INFORMATION FOR SEQUENCE ID NO: 44:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

TAATACGACT CACTATAGGG AGGACGATGC GG	32
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## (2) INFORMATION FOR SEQUENCE ID NO: 45:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4  
TCGGGCGAGT CGTCTG

## (2) INFORMATION FOR SEQUENCE ID NO: 46:

- (i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 71 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4  
GGGAGGGACGA TGCGGNNNN NNNNNNNNNN NNNNNNNNNN NNNNN  
NNNNNCAGAC GACTCGCCCG A

## (2) INFORMATION FOR SEQUENCE ID NO: 47:

- (i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 15 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4  
GGGAGGGACGA TGCAG

## (2) INFORMATION FOR SEQUENCE ID NO: 48:

- (i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4  
NNNTCGGGCG AGTCGTCTG

## (2) INFORMATION FOR SEQUENCE ID NO: 49:

- (i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 87 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4  
GCCTGTTGTG AGCCTCCTGT CGAANNNNNN NNNNNNNNNN NNNNN  
NNNNNNNNNN NNNNTTGAGC GTTTATTCTT GTCTCCC

## (2) INFORMATION FOR SEQUENCE ID NO: 50:

- (i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:  
TAATACGACT CACTATAGGG AGACAAGAAT AAACGCTCAA 40
- (2) INFORMATION FOR SEQUENCE ID NO: 51:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:  
GCCTGTTGTG AGCCTCCTGT CGAA 24
- (2) INFORMATION FOR SEQUENCE ID NO: 52:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 87 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:  
GGGAGACAAG AATAAACGCT CAANNNNNN NNNNNNNNNN NNNNNNNNNN 50  
NNNNNNNNNN NNNTTCGACA GGAGGCTCAC AACAGGC 87
- (2) INFORMATION FOR SEQUENCE ID NO: 53:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:  
GGGAGACAAG AATAAACGCT CAA 23
- (2) INFORMATION FOR SEQUENCE ID NO: 54:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: N at positions 1-3 is biotin  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:  
NNNGCCTGTT GTGAGCCTCC TGTCGAA 27
- (2) INFORMATION FOR SEQUENCE ID NO: 55:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 71 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GGGAGGACGA TGCAGCCAGG GGGGTATGG GGGTGGTGCT ACTTACTTGC	50
GTCTTCAGAC GACTCGCCCG A	71

## (2) INFORMATION FOR SEQUENCE ID NO: 56:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

GGGAGGACGA TGCAGCCAGG GGGGTATGG GGGTAGTGCT ACTTACTTGC	50
GTCTTCAGAC GACTCGCCCG A	71

## (2) INFORMATION FOR SEQUENCE ID NO: 57:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GGGAGGACGA TGCAGCCAGG GGGGTATGG GGGTAGTACT ACTTACTTAC	50
GTCTTCAGAC GACTCGCCCG A	71

## (2) INFORMATION FOR SEQUENCE ID NO: 58:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 70 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

GGGAGGACGA TGCAGCCAGG GGGGTATGG GGGTATACTA CTTACTTACG	50
TCTTCAGAC GACTCGCCCG A	70

## (2) INFORMATION FOR SEQUENCE ID NO: 59:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

GGGAGGACGA TGCAGCCAGG GGGGTATGG GGGTAATACT ACTTACTTAC	50
ATCTTCAGAC GACTCGCCCG A	71

## (2) INFORMATION FOR SEQUENCE ID NO: 60:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

120

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:  
 GGGAGGACGA TGCGGCCAGG GGGGGTATGG GGGTAATACT ACTTACTTAC 50  
 GTCTTCAGAC GACTCGCCCG A 71

## (2) INFORMATION FOR SEQUENCE ID NO: 61:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GGGAGGACGA TGCGGCCAGG GGGGGTATGG GGGTGGTGTT ACTTACTTGC 50  
 GTCTTCAGAC GACTCGCCCG A 71

## (2) INFORMATION FOR SEQUENCE ID NO: 62:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

GGGAGGACGA TGCGGCCAGG GGGGGTATGG GGGTGGTGCT TCTTACTTGC 50  
 GTCTTCAGAC GACTCGCCCG A 71

## (2) INFORMATION FOR SEQUENCE ID NO: 63:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 73 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

GGGAGGACGA TGCGGCCAGG GGGGGTATGG GGGTGGTGTA CTTTTTCCTG 50  
 CGTCTTCCAG ACGACTCGCC CGA 73

## (2) INFORMATION FOR SEQUENCE ID NO: 64:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 72 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

GGGAGGACGA TGCGGCCAGG GGGGGTATGG GGGTGGTTCG TTTTTCTTG 50  
 CGGCTTCAGA CGACTCGCCC GA 72

## (2) INFORMATION FOR SEQUENCE ID NO: 65:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:  
 GGGAGGACGA TGCAGGCCAGG GGGGGTGTGG GGGTAGGTGTA CTTTTCTTG 50  
 TCTTCAGAC GACTCGCCCG A 71

(2) INFORMATION FOR SEQUENCE ID NO: 66:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:  
 GGGAGGACGA TGCAGGCCAGG GGGGGTATGG GGGTAGGTTTG GTATGTTGCG 50  
 TCCGTCAGAC GACTCGCCCG A 71

(2) INFORMATION FOR SEQUENCE ID NO: 67:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:  
 GGGAGGACGA TGCAGGCCAGG GTGGGTATGG GGGTAATACT ACTTACTTAC 50  
 GTCTTCAGAC GACTCGCCCG A 71

(2) INFORMATION FOR SEQUENCE ID NO: 68:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 72 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:  
 GGGAGGACGA TGCAGGCCAGG GGTGGTAGG GGGTAGTGC TACTTACTTA 50  
 CGTCTTCAGAC GACTCGCCCG A 72

(2) INFORMATION FOR SEQUENCE ID NO: 69:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:  
 GGGAGGACGA TGCAGGCCAGG GTCGGTGTGG GGGTAGTACT ACTTACTTGC 50  
 GTCTTCAGAC GACTCGCCCG A 71

(2) INFORMATION FOR SEQUENCE ID NO: 70:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:  
 GGGAGGACGA TGCGGCCAGG GTGGGTATGG GGGTAGTGCT ACTTACTTGC       50  
 GTCTTCAGAC GACTCGCCCCG A   71

## (2) INFORMATION FOR SEQUENCE ID NO: 71:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:  
 GGGAGGACGA TGCGGCCAGG GTGGGTATGG GGGTAGTGCT ACTTACTTGC       50  
 GTCTTCAGAC GACTCGCCCCG A   71

## (2) INFORMATION FOR SEQUENCE ID NO: 72:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:  
 GGGAGGACGA TGCGGCCCTGG GTGGGTATGG GGGTAGTGCT ACTTACTTGC       50  
 GTCTTCAGACG ACTCGCCCCG A   71

## (2) INFORMATION FOR SEQUENCE ID NO: 73:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 72 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:  
 GGGAGGACGA TGCGGCCACG GGTGGGTGTG GGGTAGTGTC TCTCACTTTA       50  
 CATCACCAGA CGACTCGCCCC GA   72

## (2) INFORMATION FOR SEQUENCE ID NO: 74:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:  
 GGGAGGACGA TGCGGCCCGG GGTGGGTGTG GGGTAGTGTA TTATATTAC       50  
 AGCCTCAGAC GACTCGCCCCG A   71

## (2) INFORMATION FOR SEQUENCE ID NO: 75:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:
 

GGGAGGACGA TGCAGGCCAGG GTCGGTGTGG GGTGGTGTAC TTTTCCTGT	50
CCTTCAGAC GACTCGCCCG A	71
  
- (2) INFORMATION FOR SEQUENCE ID NO: 76:
  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 71 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:
 

GGGAGGACGA TGCAGGCCAGG GTCGGTATGG GGTAGTGTAC TTTTAATGA	50
TCTTCAGAC GACTCGCCCG A	71
  
- (2) INFORMATION FOR SEQUENCE ID NO: 77:
  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 72 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:
 

GGGAGGACGA TGCAGGCCCGG GGGAGAGCGG TGGTAGTGT TCTATAGTAT	50
TCTGTCAAGA CGACTCGCCC GA	72
  
- (2) INFORMATION FOR SEQUENCE ID NO: 78:
  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 71 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:
 

GGGAGGACGA TGCAGGCCAGG GGGGGTATGT TTTAAACT ACTTACTTAC	50
GTCTTCAGAC GACTCGCCCG A	71
  
- (2) INFORMATION FOR SEQUENCE ID NO: 79:
  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 72 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:
 

GGGAGGACGA TGCAGGCCAGG GAGGGTATGG GGGTGGTGT TCTAGTTTG	50
CGGCAGTCAGA CGACTCGCCC GA	72
  
- (2) INFORMATION FOR SEQUENCE ID NO: 80:
  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 72 base pairs

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- (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:
- |  |    |
|--|----|
| GGGAGGACGA TCGGGCCAGG GTGGGCATGG GGGTGGTGTG GATTAATTCT | 50 |
| TCGTCCCAGA CGACTCGCCC GA                               | 72 |
- (2) INFORMATION FOR SEQUENCE ID NO: 81:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 71 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:
- |   |    |
|---|----|
| GGGAGGACGA TCGGGCCAGG GTCGGTGTGG GGTGGTGTGTT TTATTTACTC | 50 |
| GTCGCCAGAC GACTCGCCCG A                                 | 71 |
- (2) INFORMATION FOR SEQUENCE ID NO: 82:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 71 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:
- |   |    |
|---|----|
| GGGAGGACGA TCGGGGGGGC GGCTTGAAG AGGTTGCCGG TTGGAGTATT | 50 |
| CGAGCCAGAC GACTCGCCCG A                               | 71 |
- (2) INFORMATION FOR SEQUENCE ID NO: 83:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 67 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:
- |   |    |
|---|----|
| GGGAGGACGA TCGGGCCAGG TGTGGGTGG TTTGGGTTTT CTTTCGTCGC | 50 |
| CCAGACGACT CGCCCGA                                    | 67 |
- (2) INFORMATION FOR SEQUENCE ID NO: 84:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 70 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: DNA
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:
- |  |    |
|--|----|
| GGGAGGACGA TCGGGCCAGG GTGGGTATGG GGGTTTAATT AATTCTTCGT | 50 |
| CCCACACAGACG ACTCGCCCGA                                | 70 |
- (2) INFORMATION FOR SEQUENCE ID NO: 85:
- (i) SEQUENCE CHARACTERIZATION:

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- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:  
GGGAGGACGA TGCAGGGGGC GGCTTGAAG AGGTTGCCGG TTGGAGTATT 50  
CGAGCCAGAC GACTCGCCCG A 71
  
- (2) INFORMATION FOR SEQUENCE ID NO: 86:  
 (i) SEQUENCE CHARACTERIZATION:  
  - (A) LENGTH: 72 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:  
GGGAGGACGA TGCAGGCCGG GGTGGGTGTG GGGTGGTGTG AATTAATTCT 50  
TCGTCCCAGA CGACTCGCCC GA 72
  
- (2) INFORMATION FOR SEQUENCE ID NO: 87:  
 (i) SEQUENCE CHARACTERIZATION:  
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:  
GGGAGGACGA TGCAGGCCGG GGTGGGTGTG GGGTGGTGTG TTATATTGCA 50  
GGCCTCAGAC GACTCGCCCG A 71
  
- (2) INFORMATION FOR SEQUENCE ID NO: 88:  
 (i) SEQUENCE CHARACTERIZATION:  
  - (A) LENGTH: 70 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:  
GGGAGGACGA TGCAGGCCAG GTCGGGTGTG GTGGGTGTACT TTTTCCTGTC 50  
CTTCCAGACG ACTCGCCCG A 70
  
- (2) INFORMATION FOR SEQUENCE ID NO: 89:  
 (i) SEQUENCE CHARACTERIZATION:  
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:  
GGGAGGACGA TGCAGGGGGC GGCTTGAAG AGGTTGCCGG TTGGAGTATT 50  
CGAGCCAGAC GACTCGCCCG A 71
  
- (2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:  
ATCCGCTGA TTAGCGATAAC TNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  
NNNNNNNNNN NACTTGAGCA AAATCACCTG CAGGGG 50  
86

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(ix) FEATURE:

- (D) OTHER INFORMATION: N at positions 1-3 is biotin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:  
NNNCCTGC AGGTGATTTC GCTCAAGT 28

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:  
CCGAAGCTTA ATACGACTCA CTATAGGGAT CCGCCTGATT AGCGATACT 49

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 84 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:  
ATCCGCTGA TTAGCGATAAC TAGGCTTGAC AAAGGGCACC ATGGCTTAGT 50  
GGTCCTAGTA CTTGAGCAA ATCACCTGCA GGGG 84

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:  
ATCCGCTGA TTAGCGATAAC TCAGGGCACT GCAAGCAATT GTGGTCCCAA 50  
TGGGCTGAGT ACTTGAGCAA AATCACCTGCA AGGGG 85

- |  |    |
|--|----|
| (2) INFORMATION FOR SEQ ID NO: 95:                     |    |
| (i) SEQUENCE CHARACTERIZATION:                         |    |
| (A) LENGTH: 86 base pairs                              |    |
| (B) TYPE: nucleic acid                                 |    |
| (C) STRANDEDNESS: single                               |    |
| (D) TOPOLOGY: linear                                   |    |
| (ii) MOLECULAR TYPE: DNA                               |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:              |    |
| ATCCGCCTGA TTAGCGATAC TCCAGGCACT CATGGTCATT GTTTACAGTC | 50 |
| GTGGAGTAGG TACTTGAGCA AAATCACCTG CAGGGG                | 86 |
| (2) INFORMATION FOR SEQ ID NO: 96:                     |    |
| (i) SEQUENCE CHARACTERIZATION:                         |    |
| (A) LENGTH: 85 base pairs                              |    |
| (B) TYPE: nucleic acid                                 |    |
| (C) STRANDEDNESS: single                               |    |
| (D) TOPOLOGY: linear                                   |    |
| (ii) MOLECULAR TYPE: DNA                               |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:              |    |
| ATCCGCCTGA TTAGCGATAC TAGGTGATCC CTGCAAAGGC AGGATAACGT | 50 |
| CCTGAGCATC ACTTGAGCAA AATCACCTGC AGGGG                 | 85 |
| (2) INFORMATION FOR SEQ ID NO: 97:                     |    |
| (i) SEQUENCE CHARACTERIZATION:                         |    |
| (A) LENGTH: 83 base pairs                              |    |
| (B) TYPE: nucleic acid                                 |    |
| (C) STRANDEDNESS: single                               |    |
| (D) TOPOLOGY: linear                                   |    |
| (ii) MOLECULAR TYPE: DNA                               |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:              |    |
| ATCCGCCTGA TTAGCGATAC TATGTGATCC CTGCAGAGGG AGGANACGTC | 50 |
| TGAGCATCAC TTGAGCAAAA TCACCTGCAG GGG                   | 83 |
| (2) INFORMATION FOR SEQ ID NO: 98:                     |    |
| (i) SEQUENCE CHARACTERIZATION:                         |    |
| (A) LENGTH: 86 base pairs                              |    |
| (B) TYPE: nucleic acid                                 |    |
| (C) STRANDEDNESS: single                               |    |
| (D) TOPOLOGY: linear                                   |    |
| (ii) MOLECULAR TYPE: DNA                               |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:              |    |
| ATCCGCCTGA TTAGCGATAC TCACGTGATC CCATAAGGGC TGCGAAAT   | 50 |
| AGCAGAGCAT CACTTGAGCA AAATCACCTG CAGGGG                | 86 |
| (2) INFORMATION FOR SEQ ID NO: 99:                     |    |
| (i) SEQUENCE CHARACTERIZATION:                         |    |
| (A) LENGTH: 86 base pairs                              |    |
| (B) TYPE: nucleic acid                                 |    |
| (C) STRANDEDNESS: single                               |    |
| (D) TOPOLOGY: linear                                   |    |
| (ii) MOLECULAR TYPE: DNA                               |    |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:              |    |
| ATCCGCCTGA TTAGCGATAC TGGTGGACTA GAGGGCAGCA AACGATCCTT | 50 |
| GGTTAGCGTC CACTTGAGCA AAATCACCTG CAGGGG                | 86 |

## (2) INFORMATION FOR SEQ ID NO: 100:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

ATCCGCCTGA TTAGCGATAAC TGGTGCGACG AGGCTTACAC AAACGTACAC  
GTTTCCCCGC ACTTGAGCAA AATCACCTGC AGGGG

50

85

## (2) INFORMATION FOR SEQ ID NO: 101:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

ATCCGCCTGA TTAGCGATAAC TTGTCGGAGC AGGGGCGTAC GAAAACTTA  
CAGTTCCCCC GACTTGAGCA AAATCACCTG CAGGGG

50

86

## (2) INFORMATION FOR SEQ ID NO: 102:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

ATCCGCCTGA TTAGCGATAAC TAGTGGAAACA GGGCACGGAG AGTCAAACCTT  
TGGTTTCCCC CACTTGAGCA AAATCACCTG CAGGGG

50

86

## (2) INFORMATION FOR SEQ ID NO: 103:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

ATCCGCCTGA TTAGCGATAAC TGTGGGTAGG GATCGGTGGA TGCCTCGTCA  
CTTCTAGTCC CACTTGAGCA AAATCACCTG CAGGGG

50

86

## (2) INFORMATION FOR SEQ ID NO: 104:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

ATCCGCCTGA TTAGCGATAAC TGGGCGCCCT AAACAAAGGG TGGTCACTTC

50

TAGTCCCAGG AACTTGAGCA AAATCACCTG CAGGGG

86

## (2) INFORMATION FOR SEQ ID NO: 105:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 87 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

ATCCGCCTGA TTAGCGATAC TTCCGGGCTC GGGATTCTGTG GTCACTTTCA  
GTCCCCGATA TAACTTGAGC AAAATCACCT GCAGGGG

50

87

## (2) INFORMATION FOR SEQ ID NO: 106:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 84 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

ATCCGCCTGA TTAGCGATAC TATGGGAGGG CGCGTTCTTC GTGGTTACTT  
TTAGTCCCGA CTTGAGCAAA ATCACCTGCA GGGG

50

84

## (2) INFORMATION FOR SEQ ID NO: 107:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 84 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

ATCCGCCTGA TTAGCGATAC TACGGGAGGG CACGTTCTTC GTGGTTACTT  
TTAGTCCCGA CTTGAGCAAA ATCACCTGCA GGGG

50

84

## (2) INFORMATION FOR SEQ ID NO: 108:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

ATCCGCCTGA TTAGCGATAC TGCTCGTAGG GGGCGATTCT TTCGCCGTTA  
CTTCCAGTCC TACTTGAGCA AAATCACCTG CAGGGG

50

86

## (2) INFORMATION FOR SEQ ID NO: 109:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

ATCCGCCTGA TTAGCGATAACATGAGC ATCGTCTCAC	50
GATCCTCAGCA CACTTGAGCA AAATCACCTG CAGGGG	86

## (2) INFORMATION FOR SEQ ID NO: 110:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

ATCCGCCTGA TTAGCGATACTCCACAGGCT ACGGCACGTA GAGCATCACC	50
ATGATCCTGT GACTTGAGCA AAATCACCTG CAGGGG	86

## (2) INFORMATION FOR SEQ ID NO: 111:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

ATCCGCCTGA TTAGCGATACTGCAGGCTG GCACATGAGC ATCTCTGATC	50
CCGCAATCCT CACTTGAGCA AAATCACCTG CAGGGG	86

## (2) INFORMATION FOR SEQ ID NO: 112:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

ATCCGCCTGA TTAGCGATACTACGGGCTA CTTCTAGAG CATCTCTGAT	50
CCCGGTGCTC GACTTGAGCA AAATCACCTG CAGGGG	86

## (2) INFORMATION FOR SEQ ID NO: 113:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

ATCCGCCTGA TTAGCGATACTAAAGGGCGA ACGTAGGTCTG AGGCATCCAT	50
TGGATCCCTT CACTTGAGCA AAATCACCTG CAGGGG	86

## (2) INFORMATION FOR SEQ ID NO: 114:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:  
 ATCCGCCTGA TTAGCGATAC TACGGGCTCT GTCACTGTGG CACTAGCAAT 50  
 AGTCCCCTCG CACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO: 115:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 85 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:  
 ATCCGCCTGA TTAGCGATAC TGGGCAGACC TTCTGGACGA GCATCACCTA 50  
 TGTGATCCCC ACTTGAGCAA AAATCACCTG CAGGGG 85

(2) INFORMATION FOR SEQ ID NO: 116:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 86 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:  
 ATCCGCCTGA TTAGCGATAC TAGAGGGGAA GTAGGGCTGCC TGACTCGAGA 50  
 GAGTCCTCCC GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO: 117:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 86 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:  
 ATCCGCCTGA TTAGCGATAC TAGGGGTGCG AAACACATAA TCCTCGCGGA 50  
 TTCCCATCGC TACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO: 118:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 83 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:  
 ATCCGCCTGA TTAGCGATAC TGGGGGGGCA ATGGCGGTAC CTCTGGTCCC 50  
 CTAAATACAC TTGAGCAAAA TCACCTGCAG GGG 83

(2) INFORMATION FOR SEQ ID NO: 119:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 85 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

- (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:  
 ATCCGCCTGA TTAGCGATAC TGCGGCTCAA AGTCCTGCTA CCCGCAGCAC 50  
 ATCTGTGGTC ACTTGAGCAA AATCACCTGC AGGGG 85
- (2) INFORMATION FOR SEQ ID NO: 120:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 86 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:  
 ATCCGCCTGA TTAGCGATAC TTTGGGCGTG AATGTCCACG GGTACCTCCG 50  
 GTCCCCAAAGA GACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO: 121:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 85 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:  
 ATCCGCCTGA TTAGCGATAC TTCCGCGCAA GTCCCTGGTA AAGGGCAGCC 50  
 CTAACTGGTC ACTTGAGCAA AATCACCTGC AGGGG 85
- (2) INFORMATION FOR SEQ ID NO: 122:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 86 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:  
 ATCCGCCTGA TTAGCGATAC TCAAGTTCCC CACAAGACTG GGGCTGTTCA 50  
 AACCGCTAGT AACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO: 123:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 86 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single  
   (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:  
 ATCCGCCTGA TTAGCGATAC TCAAGTAGGG CGCGACACAC GTCCGGGCAC 50  
 CTAAGGTCCC AACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO: 124:  
 (i) SEQUENCE CHARACTERIZATION:  
   (A) LENGTH: 86 base pairs  
   (B) TYPE: nucleic acid  
   (C) STRANDEDNESS: single

(D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: DNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:  
 ATCCGCTGATAGCGATAC TAAAGTCGTG CAGGGTCCCC TGGAAGCATC 50  
 TCCGATCCA GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO: 125:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 96 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:  
 GGGAGACAAG AAUAACGCUC AANNNNNNNN NNNNNNNNNN NNNNNNNNNN 50  
 NNNNNNNNNN NNNNNNNNNN NNUUCGACAG GAGGCUCACA ACAGGC 96

(2) INFORMATION FOR SEQ ID NO: 126:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 39 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:  
 TAATACGACT CACTATAGGG AGACAAGAAT AACGCTCAA 39

(2) INFORMATION FOR SEQ ID NO: 127:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:  
 GCCTGTTGTG AGCTCCTGT CGAA 24

(2) INFORMATION FOR SEQ ID NO: 128:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 93 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:  
 GGGAGACAAG AAUAACGCUC AACGGUGGCA UUUCUUCACU UCCUUCUCGC 50  
 UUUCUCGCGU UGGGCNGAU UCGACAGGAG GCUCACAACA GGC 93

(2) INFORMATION FOR SEQ ID NO: 129:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 91 base pairs  
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:  
 GGGAGACAAG AAUAACGCUC AACCAACCUU CUGUCGGCGU UGCUUUUJUGG       50  
 ACGGCACUCA GGCUCCAUUC GACAGGAGGC UCACAAACAGG C               91

(2) INFORMATION FOR SEQ ID NO:130:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 95 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:130::  
 GGGAGACAAG AAUAACGCUC AAUCGAUCGG UUGUGUGCCG GACAGCCUUA       50  
 ACCAGGGCUG GGACCGAGGC CUUCGACAGG AGGCUCACAA CAGGC           95

(2) INFORMATION FOR SEQ ID NO: 131:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 92 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:  
 GGGAGACAAG AAUAACGCUC AACUGAGUAG GGGAGGAAGU UGAAUCAGUU       50  
 GUGGCGCCUC UCAUUCGCUU CGACAGGAGG CUCACAAACAG GC           92

(2) INFORMATION FOR SEQ ID NO: 132:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 95 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132:  
 GGGAGACAAG AAUAACGCUC AACAGCACUU UCGCUUUUCA UCAUUUUUUC       50  
 UUJUCCACUGU UGGGCAGGA AUUCGACAGG AGGCUCACAA CAGGC           95

(2) INFORMATION FOR SEQ ID NO: 133:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 96 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear



- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:
- |  |    |
|--|----|
| GGGAGACAAG AAAAACGCUC AAGUGCAAAC UUJACCCGGG AACCGCGCGU | 50 |
| UUCGAUCGAC UUUCCUUUCC AUUCGACAGG AGGCUCACAA CAGGC      | 95 |
- (2) INFORMATION FOR SEQ ID NO: 138:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 96 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138:
- |  |    |
|--|----|
| GGGAGACAAG AAAAACGCUC AAAUUCCGCG UUCCGAUUAU ACCUGUGCUC | 50 |
| GGAAAUCGGU AGCCAUAGUG CAUUCGACAG GAGGCUCACA ACAGGC     | 96 |
- (2) INFORMATION FOR SEQ ID NO: 139:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 94 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:
- |   |    |
|---|----|
| GGGAGACAAG AAAAACGCUC AACGAACGAG GAGGGAGUGG CAAGGGAUJGG | 50 |
| UUGGAUAGGC UCUACGCUCA UUCGACAGGA GGCUCACAAAC AGGC       | 94 |
- (2) INFORMATION FOR SEQ ID NO: 140:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 94 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:
- |   |    |
|---|----|
| GGGAGACAAG AAAAACGCUC AAGCGAACU GGCGACUUGC UCCAUJGGCC | 50 |
| GAUAAUACGA UUCGGUCAU UUCGACAGGA GGCUCACAAAC AGGC      | 94 |
- (2) INFORMATION FOR SEQ ID NO: 141:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 95 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141:

GGGAGACAAG AAUAACGCUC AACGAACGAG GAGGGAGUCC CAAGGGAUCC	50
UUGGAAUAGGC UCUACGCUC AAUUCGACAGG AGGCUCACAA CAGGC	95

## (2) INFORMATION FOR SEQ ID NO: 142:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:

GGGAGACAAG AAUAACGCUC AACGAGAACU GACUACGAUG ACGAAGGCCG	50
CGGGUJUGAAU CCCUCAUUGA UUCGACAGGA GGCUCACAAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 143:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 95 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:

GGGAGACAAG AAUAACGCUC AAAAGCAACG AGACCUGACG CCUGAUGUGA	50
CUGUGCUJUGC ACCCGAUUCU GUUCGACAGG AGGCUCACAA CAGGC	95

## (2) INFORMATION FOR SEQ ID NO: 144:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:

GGGAGACAAG AAUAACGCUC AAGUGAUUCU CAUUCUCAAU GCUUUCUCAC	50
AACUUUUUJUCC ACUUCAGCGU GAUUCGACAG GAGGCUCACAA ACAGGC	96

## (2) INFORMATION FOR SEQ ID NO: 145:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:

GGGAGACAAG AAUAACGCUC AAAAGCAACG AGACUCGACG CCUGAUGUGA	50
CUGUGCUJUGC ACCCGAUUCU UUCGACAGGA GGCUCACAAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 146:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:

GGGAGACAAG AAUAACGCUC AAUCGAUCGG UUGUGUGCCG GACAGCUUJUG	50
ACCAUGAGCU GGGACCGAGG CCUUCGACAG GAGGCUCACA ACAGGC	96

## (2) INFORMATION FOR SEQ ID NO: 147:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:

GGGAGACAAG AAUAACGCUC AANGACGNLU GGACCUGACU AAUCGACUGA	50
UCAAAGAUCC CGCCCCAGAUG GGUUCGACAG GAGGCUCACA ACAGGC	96

## (2) INFORMATION FOR SEQ ID NO: 148:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 148:

GGGAGACAAG AAUAACGCUC AACACUGCGA CUUGCAGAAG CCUUGUGUGG	50
CGGUACCCCC UUJUGGCCUCG UUCGACAGGA GGCUCACAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 149:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

GGGAGACAAG AAUAACGCUC AAGGUGGCAU UUCUUCAUUU UCCUUCUCGC	50
UUUCUCCGCC GUUGGGCGCG UUCGACAGGA GGCUCACAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 150:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:

GGGAGACAAG AAUAACGCUC AACUGAGUA GGGGGAAAG UUGAAUCAGU	50
UGUGGGCGCUC UACUCAUUCG CCUUCGACAG GAGGCUCACA ACAGGC	96

## (2) INFORMATION FOR SEQ ID NO: 151:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:

GGGAGACAAG AAUAACGCUC AAGUCGAAAC UGGCGACUUG CUCCAUUGGC	50
CGAUUAACG AUUCGGGUCA UUUCGACAGGA GGCUCACAAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 152:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:

GGGAGACAAG AAUAACGCUC AAGCGAUACU GGCACUUGC UCCAUUGGCC	50
GAUUAACGA UUCGGCUCAG UUUCGACAGGA GGCUCACAAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 153:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:

GGGAGACAAG AAUAACGCUC AACUGGGGG CACAGGACCG AGAGUCCCUC	50
CGGCAAUAGC CGCUACCCCA CCUUCGACAG GAGGCUCACA ACAGGC	96

## (2) INFORMATION FOR SEQ ID NO: 154:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:

GGGAGACAAG AAUAACGCUC AACACAGCCU NANAGGGGGG AAGUUGAAUC	50
AGUUUGUGGCG CUCUACUCAU UCGCUUCGAC AGGAGGCUA CAACAGGC	98

## (2) INFORMATION FOR SEQ ID NO: 155:

- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 155:

GGGAGACAAG AAUAACGCUC AAANGGNNUA UGGUGACUUG CUCCAUUGGC	50
CGAUUAACG AUUCGGUCAG UUCGACAGGA GGCUCACAAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 156:

- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 156:

GGGAGACAAG AAUAACGCUC AACCUUGCGUA GGGNGGGAAG UUGAAUCAGU	50
UGUGGCGCUC UACUCAUUCG CCUUCGACAG GAGGCUCACAA ACAGGC	96

## (2) INFORMATION FOR SEQ ID NO: 157:

- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 94 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-F modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 157:

GGGAGACAAG AAUAACGCUC AACGAACGAG GAGGGAGUGG CAAGGGAUGG	50
UUGGAUAGGC UCUACGCUCA UUCGACAGGA GGCUCACAAAC AGGC	94

## (2) INFORMATION FOR SEQ ID NO: 158:

- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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- (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 158:
- |  |    |
|--|----|
| GGGAGACAAG AAUAACGCUC AAGUGCAAAC UUAACCCGGG AACCGCGCGU | 50 |
| UUCGAUUCGC UUUCNUAUU CCAUUCGACA GGAGGCUCAC AACAGGC     | 97 |
- (2) INFORMATION FOR SEQ ID NO: 159:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 93 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 159:
- |  |    |
|--|----|
| GGGAGACAAG AAUAACGCUC AACGAACGAG GAGGGAGUGG CAAGGGACGG | 50 |
| UNNAUAGGCU CUACGCUCAU UCGACAGGGAG GCUCACAACA GGC       | 93 |
- (2) INFORMATION FOR SEQ ID NO: 160:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 93 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 160:
- |  |    |
|--|----|
| GGGAGACAAG AAUAACGCUC AAUCGGUGUG GCUCAGAAAC UGACACGCGU | 50 |
| GAGCUUCGCA CACAUCAUGCU UCGACAGGGAG GCUCACAACA GGC      | 93 |
- (2) INFORMATION FOR SEQ ID NO: 161:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 95 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 161:
- |  |    |
|--|----|
| GGGAGACAAG AAUAACGCUC AAUAUCGCUU UUCAUCAAUU CCACUUUUUC | 50 |
| ACUCUNUAAC UUGGGCGUGC AUUCGACAGG AGGCUCACAA CAGGC      | 95 |
- (2) INFORMATION FOR SEQ ID NO: 162:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 96 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 162:

GGGAGACAAG AAUAACGCUC AAGUGCAAAC UUAACCCGGG AACCGCGCGU	50
UUCGAUCCUG CAUCCUUUUU CCUUCGACAG GAGGCUCACCA ACAGGC	96

## (2) INFORMATION FOR SEQ ID NO: 163:

## (i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 93 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 163:

GGGAGACAAG AAUAACGCUC AAUCGNUCGG UUGUGUGCCG GCAGCUUJUGU	50
CCAGCGUUGG CCCGAGGCCU UCGACAGGAG GCUCACAAACA GGC	93

## (2) INFORMATION FOR SEQ ID NO: 164:

## (i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 95 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 164:

GGGAGACAAG AAUAACGCUC AAAGUACCCA UCUCAUUUU UCCUUUCCUU	50
UCUUCAAGGC ACAUUGAGGG UUUCGACAGG AGGCUCACAA CAGGC	95

## (2) INFORMATION FOR SEQ ID NO: 165:

## (i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 97 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-F modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 165:

GGGAGACAAG AAUAACGCUC AACUGAGUA GGGGGGGAAG UUGAACCAAGU	50
UGUGGCGNGCC UACUCAUCN CCAUUCGACA GGAGGCUCAC AACAGGC	97

## (2) INFORMATION FOR SEQ ID NO: 166:

## (i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 94 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-F modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 166:  
 GGGAGACAAG AAUAACGCUC AACCNCCUN CUGUCGGCGC UUGUCUUUUU 50  
 GGACGGCAA CCCAGGGCUC UUCGACAGGA GGCUCACAAC AGGC 94

(2) INFORMATION FOR SEQ ID NO: 167:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 96 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 167:  
 GGGAGACAAG AAUAACGCUC AACCAACCUN CUGUCGGCGC UUGUCUUUUU 50  
 GGACGAGCAA CUCAAGGCUC GUUUCGACAG GAGGCUCACA ACAGGC 96

(2) INFORMATION FOR SEQ ID NO: 168:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 93 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 168:  
 GGGAGACAAG AAUAACGCUC AACCAGCGCA GAUCCCGGGC UGAAGUGACU 50  
 GCCGGCAACG GCCGUCCAU UCGACAGGAG GCUCACAACA GGC 93

(2) INFORMATION FOR SEQ ID NO: 169:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 96 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 169:  
 GGGAGACAAG AAUAACGCUC AAUUCCGUA ACAACUUUJC AUJJUUCACUU 50  
 UUCAUCCAAC CAGUGAGCAG CAUUCGACAG GAGGCUCACA ACAGGC 96

(2) INFORMATION FOR SEQ ID NO: 170:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 96 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-F modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 170:  
 GGGAGACAAG AAUAACGCUC AAUAUCGCUU UCAUCAAAUJ CCACUCCUJC 50

ACUUUCUUUA CUUGGGCGUG CAUUCGACAG GAGGCUCACA ACAGGC

96

## (2) INFORMATION FOR SEQ ID NO: 171:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: N at positions 1 and 23  
base pair to each other and can be 1-4  
base pairs long.

## (ix) FEATURE:

- (D) OTHER INFORMATION: N at positions 5 and 10  
is any base pair.

## (ix) FEATURE:

- (D) OTHER INFORMATION: N at positions 6 and 9  
is any base pair.

## (ix) FEATURE:

- (D) OTHER INFORMATION: N at positions 7 and 8 is  
any base pair.

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 171:

NGGCNNNNNN GRKYAYYRRT CCN

23

## (2) INFORMATION FOR SEQ ID NO: 172:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: Nucleotide 38 is an  
inverted orientation T (3'-3' linked)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 172:

TGGGAGGGCG CGTTCTTCGT GGTTACTTTT AGTCCCGT

38

## (2) INFORMATION FOR SEQ ID NO: 173:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: Nucleotide 40 is an  
inverted orientation T (3'-3' linked)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 173:

CACAGGCTAC GGCACGTAGA GCATCACCAT GATCCTGTGT

40

## (2) INFORMATION FOR SEQ ID NO: 174:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: Nucleotide 45 is an inverted orientation T (3'-3' linked)  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 174:  
TACTCAGGGC ACTGCAAGCA ATTGTGGTCC CAATGGGCTG AGTAT

45

(2) INFORMATION FOR SEQ ID NO: 175:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: C at positions 8, 11, 25 and 26 is 2'-O-methyl-2'-deoxycytidine.  
(ix) FEATURE:  
(D) OTHER INFORMATION: G at positions 9, 10, 17, 19 and 35 is 2'-O-methyl-2'-deoxyguanosine.  
(ix) FEATURE:  
(D) OTHER INFORMATION: A at positions 12, 24 and 27 is 2'-O-methyl-2'-deoxyadenosine.  
(ix) FEATURE:  
(D) OTHER INFORMATION: U at position 34 is 2'-O-methyl-2'-deoxyuridine.  
(ix) FEATURE:  
(D) OTHER INFORMATION: U at positions 6 and 22 is 2'-fluoro-2'-deoxyuridine.  
(ix) FEATURE:  
(D) OTHER INFORMATION: C at positions 23, 32 and 33 is 2'-fluoro-2'-deoxycytidine.  
(ix) FEATURE:  
(D) OTHER INFORMATION: Nucleotide 36 is an inverted orientation T (3'-3' linked).  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 175:

CAGGUACGG CACGTAGAGC AUCACCATGA TCCUGT

36

(2) INFORMATION FOR SEQ ID NO: 176:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: DNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: C at position 8 is 2'-O-methyl-2'-deoxycytidine.  
(ix) FEATURE:  
(D) OTHER INFORMATION: G at positions 9, 17 and 31 is 2'-O-methyl-2'-deoxyguanosine.

- (ix) FEATURE:
  - (D) OTHER INFORMATION: A at position 22 is 2'-O-methyl-2'-deoxyadenine.
- (ix) FEATURE:
  - (D) OTHER INFORMATION: U at position 30 is 2'-O-methyl-2'-deoxyuridine.
- (ix) FEATURE:
  - (D) OTHER INFORMATION: U at positions 6 and 20 is 2'-fluoro-2'-deoxyuridine.
- (ix) FEATURE:
  - (D) OTHER INFORMATION: C at positions 21, 28 and 29 is 2'-fluoro-2'-deoxycytidine.
- (ix) FEATURE:
  - (D) OTHER INFORMATION: N at positions 10 and 23 is pentaethylene glycol phosphoramidite spacer.
- (ix) FEATURE:
  - (D) OTHER INFORMATION: Nucleotide 32 is an inverted orientation T (3'-3' linked)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 176:

CAGGCUACGN CGTAGAGCAU CANTGATCCU GT

32

(2) INFORMATION FOR SEQ ID NO: 177:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 39 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA
- (ix) FEATURE:
  - (D) OTHER INFORMATION: Nucleotide 39 is an inverted orientation T (3'-3' linked)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 177:

CAGTCGGTGG TAGGGCAGGT TGGGGTGACT TCGTGGAAT

39

(2) INFORMATION FOR SEQ ID NO: 178:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 37 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA
- (ix) FEATURE:
  - (D) OTHER INFORMATION: T at positions 13, 14, 16 and 17 is substituted with IdU.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 178:

TGGGAGGGCG CGTTCTTCGT GGTTACTTT AGTCCCG

37

(2) INFORMATION FOR SEQ ID NO: 179:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 37 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(ix) FEATURE:

(D) OTHER INFORMATION: T at position 20 is substituted with IdU.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 179:

TGGGAGGGCG CGTTCTTCGT GGTTACTTTT AGTCCCG

37

(2) INFORMATION FOR SEQ ID NO: 180:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(ix) FEATURE:

(D) OTHER INFORMATION: T at position 23 is substituted with IdU.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 180:

TGGGAGGGCG CGTTCTTCGT GGTTACTTTT AGTCCCG

37

(2) INFORMATION FOR SEQ ID NO: 181:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(ix) FEATURE:

(D) OTHER INFORMATION: T at position 24 is substituted with IdU.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 181:

TGGGAGGGCG CGTTCTTCGT GGTTACTTTT AGTCCCG

37

(2) INFORMATION FOR SEQ ID NO: 182:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(ix) FEATURE:

(D) OTHER INFORMATION: T at position 27 is substituted with IdU.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 182:

TGGGAGGGCG CGTTCTTCGT GGTTACTTTT AGTCCCG

37

(2) INFORMATION FOR SEQ ID NO: 183:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(ix) FEATURE:

(D) OTHER INFORMATION: T at positions 28-30 is substituted with IdU.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 183:

TGGGAGGGCG CGTTCTTCGT GGTTACTTTT AGTCCCG

37

## (2) INFORMATION FOR SEQ ID NO: 184:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: DNA

## (ix) FEATURE:

(D) OTHER INFORMATION: T at position 33 is substituted with IdU.

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 184:

TGGGAGGGCG CGTTCTTCGT GGTTACTTT AGTCCCG

37

## (2) INFORMATION FOR SEQ ID NO: 185:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: Peptide

## (ix) FEATURE:

(D) OTHER INFORMATION: Xaa at position 5 is a modified amino acid  
that could not be identified.

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 185:

Lys Lys Pro Ile Xaa Lys Lys

5

## (2) INFORMATION FOR SEQ ID NO: 186:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 186:

GGGAGGACGA UGGGGNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN  
NNNNNCAGAC GACUCGCCCG A

50

71

## (2) INFORMATION FOR SEQ ID NO: 187:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 187:

TAATACGACT CACTATAGGG AGGACGATGC GG

32

## (2) INFORMATION FOR SEQ ID NO: 188:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 188:  
 TCGGGCGAGT CGTCTG 16

(2) INFORMATION FOR SEQ ID NO: 189:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 189:  
 GGGAGGACGA UGCAGGGAGG GACGAUAAAG AGGAAUCGAA CAACAAGUGG 50  
 CUGGCCAGAC GACUCGCCCG A 71

(2) INFORMATION FOR SEQ ID NO: 190:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 190:  
 GGGAGGACGA UGCAGGGCGGG AAGGUCCGAA GACCAGCGAA AGGAACGAGA 50  
 UUGCCCAGAC GACUCGCCCG A 71

(2) INFORMATION FOR SEQ ID NO: 191:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 70 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 191:  
 GGGAGGACGA UGCAGGGUGGU GAAGAGGUAC CGGAAUUGC AAGAUACCA 50  
 GGCCCAGACG ACUCGCCCG A 70

(2) INFORMATION FOR SEQ ID NO: 192:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 192:  
 GGGAGGACGA UGCAGGGCAGG GAGCAAUGAA CUCAAGUCAA GCCGGUGCAC 50

150

GUGGGCAGAC GACUCGCCG A

71

## (2) INFORMATION FOR SEQ ID NO:193:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

GGGAGGACGA UGGGGUAGCU GCUGUCAUGC AAGACACUAG AAGAUUAAGA

50

UGGGGCAGAC GACUCGCCG A

71

## (2) INFORMATION FOR SEQ ID NO:194:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 74 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

GGGAGGACGA UGGGGGGGCC GGAAUUGAAC CGACGACUUC GGGUUAUGAG

50

CCCGACGUCA GACGACUCGC CCGA

74

## (2) INFORMATION FOR SEQ ID NO: 195:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 195:

GGGAGGACGA UGGGGUCCAG GGAAUUGAAGU GUCCCCGUAG GAACAUAAAG

50

GCGGCCAGAC GACUCGCCG A

71

## (2) INFORMATION FOR SEQ ID NO: 196:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 196:

GGGAGGACGA UGGGGAAGUU CUAACAAGUU AGUGGAAGGU UCCACUUGAA

50

UGUACAGACG ACUCGCCG A

70

- (2) INFORMATION FOR SEQ ID NO: 197:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 44 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 197:  
 GGGAGGACGA UGCCGAUGGA GCUGAAUCA GACGACUCGC CCGA

44

- (2) INFORMATION FOR SEQ ID NO: 198:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 71 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 198:  
 GGGAGGACGA UGCCGGGUGGG AAGAUGAGCC GGUCGGCAGU AAUGUGACAC  
 UGCAGAC GACUCGCCG A

50

71

- (2) INFORMATION FOR SEQ ID NO: 199:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 70 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 199:  
 GGGAGGACGA UGCCGGGAGGG AAUGAGGAAA CAACUAGCAG AUAAACCGAGC  
 UGGCCAGACG ACUCGCCG A

50

70

- (2) INFORMATION FOR SEQ ID NO: 200:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 44 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 200:  
 GGGAGGACGA UGCCGAUGGA GCUGAAUCA GACGACUCGC CCGA

44

- (2) INFORMATION FOR SEQ ID NO: 201:

- (i) SEQUENCE CHARACTERIZATION:
  - (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 201:

GGGAGGACGA UGCGGUUGCU CUACAAUGAC GCGGUGACUC CGCAGUUCUU 50  
GGACACAGAC GACUCGCCCG A 71

(2) INFORMATION FOR SEQ ID NO: 202:

(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 71 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 202:

GGGAGGACGA UGCGGGAGGG GAGAAGAAUG CAGGAAACAG CGAAAUGCUG 50  
GUGGCCAGAC GACUCGCCCG A 71

(2) INFORMATION FOR SEQ ID NO: 203:

(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 71 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 203:

GGGAGGACGA UGCGGGCGGG AAGAGCUAAU GGAAGUGGAA UCAGUCACAG 50  
UGCGGGCAGAC GACUCGCCCG A 71

(2) INFORMATION FOR SEQ ID NO: 204:

(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 204:

GGGAGGACGA UGCGGGCUUA GGGAAUUGGU UCUGAGGUGG UCAGACGACU 50  
CGCCCCGA 57

(2) INFORMATION FOR SEQ ID NO: 205:

(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 71 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 205:

GGGAGGACGA UGCAGGAAGG AACAGGAUA AGACAAGUCG AACAAAGCCG	50
AGGUGCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 206:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 44 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 206:

GGGAGGACGA UGCAGGAUGGA GCUGAAAUCG GACGACUCGC CCGA	44
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(2) INFORMATION FOR SEQ ID NO: 207:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 207:

GGGAGGACGA UGCAGGGAGA CGUAGACGGG AACAUAGAAC GAAACAUCAAC	50
GCGGCCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 208:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 69 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 208:

GGGAGGACGA UGCAGGAAGU GGAAUAGAAC GUCAGAAAUG UAAGCGUGAG	50
GUGCAGACGA CUCGCCCGA	69

(2) INFORMATION FOR SEQ ID NO: 209:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 209:

GGGAGGACGA UGCCGGAAAGG GUAGGAAGGU CAAGAGGAAA CAGCGCUUCG	50
GGGUGCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 210:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 210:

GGGAGGACGA UGCCGGGGCAA AGGAAGUUGG AAUCGGGACU AAGUAGUGUG	50
UGGCCAGACG ACUCGCCCGA	70

(2) INFORMATION FOR SEQ ID NO: 211:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 211:

GGGAGGACGA UGCCGGAGAAC CAACAGAGCC CCCUGGUGGU GGGGGAAGGA	50
UUCUCAGACG ACUCGCCCGA	70

(2) INFORMATION FOR SEQ ID NO: 212:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 212:

GGGAGGACGA UGCCGGACACA CAAGUGAAGG UCAGACGCGA AUUACGUGGG	50
UGGGCAGACG ACUCGCCCGA	70

(2) INFORMATION FOR SEQ ID NO: 213:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 82 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 213:

GGGAGGACGA UGCCGGUCGUG GGGUGGGUGG GGGCAGCGUU GGAAUAAGUA	50
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ACUGGUAAACG GCUGGCCAGA CGACUCGCCA GA

82

## (2) INFORMATION FOR SEQ ID NO: 214:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 214:

GGGAGGACGA UGCAGGGUAGG GUGGUUACCU GUAAUUUAU UGAUUCUGGC  
UUUAGCAGAC GACUCGCCCG A

50

71

## (2) INFORMATION FOR SEQ ID NO: 215:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 215:

GGGAGGACGA UGCAGGGCCCCU UAGCUCAGUG GUUAGAGCAG ACGACUCGCC  
CGA

50

53

## (2) INFORMATION FOR SEQ ID NO: 216:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 74 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 216:

GGGAGGACGA UGCAGGGUACG UGGAAUAGGG UUAAACAGCU GGAAAUAAACG  
UAGGUGGCCA GACGACUCGC CCGA

50

74

## (2) INFORMATION FOR SEQ ID NO: 217:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 217:

GGGAGGACGA UGCAGGGUAGG GAGUAGGACA GACAUAAACAG UGCAACCAUC  
GUGGCCAGAC GACUCGCCCG A

50

71

## (2) INFORMATION FOR SEQ ID NO: 218:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 218:

GGGAGGACGA UGCCGAAACG GCGUGGCCAA AGUGAGGGGG UAGGAUGUAC	50
CAUAGGUCAG ACGACUCGCC CGA	73

## (2) INFORMATION FOR SEQ ID NO: 219:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 219:

GGGAGGACGA UGGGGGAGGG GAAAAUGAGA CCGACAGAUU GACGGAAGUA	50
CUGGGCAGAC GACUCGCCCG A	71

## (2) INFORMATION FOR SEQ ID NO: 220:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 220:

GGGAGGACGA UGGGGCAUU CGUAAUACC UUGUUUUAUU CCUUUUUCUAG	50
CGGCCAGAC GACUCGCCCG A	71

## (2) INFORMATION FOR SEQ ID NO: 221:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 221:

GGGAGGACGA UGGGAUCGU AAUCGCCACU ACUACUUUCC GAACCCGCAC	50
GUGGCCAGAC GACUCGCCCG A	71

- (2) INFORMATION FOR SEQ ID NO: 222:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 70 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 222:  
 GGGAGGACGA UGCAGCGUCC CGAGUCACGC UGUCCUGAU ACCUUCUUG  
 UGCCAGACG ACUCGCCCGA 50  
 70
- (2) INFORMATION FOR SEQ ID NO:223:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 72 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 223:  
 GGGAGGACGA UGCAGGAUCC UUUGUGGGCU CUUGUUGACC CCCUCGUUGU  
 CCCCCCAGA CGACUCGCCCGA 50  
 72
- (2) INFORMATION FOR SEQ ID NO: 224:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 71 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 224:  
 GGGAGGACGA UGCAGCGGGU ACUCUUUCGCC AGCUCCUCCA AGCGCGACCU  
 GUGCCAGAC GACUCGCCCGA 50  
 71
- (2) INFORMATION FOR SEQ ID NO: 225:
- (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 72 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 225:  
 GGGAGGACGA UGCAGGUUCG AAUAGGGCCA UUUUCACUA GCUAUCCUAC 50

CCUGCCCAGA CGACUCGCC 50

72

## (2) INFORMATION FOR SEQ ID NO: 226:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 226:

GGGAGGGACGA UGCAGAUAAA GGCUAGAACU AGCUCGCAUC UUGGGUGUCCG	50
GUGCCCAGAC GACUCGCCCG A	71

## (2) INFORMATION FOR SEQ ID NO: 227:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 227:

GGGAGGGACGA UGCAGGGACCA GAUGGGCGAU UUUUCAGCAA UCCUCCCCCG	50
CUGCCCAGAC GACUCGCCCG A	71

## (2) INFORMATION FOR SEQ ID NO: 228:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 228:

GGGAGGGACGA UGCAGGUGAUG GCGACCAGUC AAACCGGUGC UUUUACUCCC	50
CCGCCAGACG ACUCGCCCGA	70

## (2) INFORMATION FOR SEQ ID NO: 229:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 229:  
 GGGAGGACGA UGCAGGGAAAU AACAGGGCCA GAUUCUCAU CUNNCUUC  
 GUGACCCAGA CGACUCGCCA GA 50  
 72
- (2) INFORMATION FOR SEQ ID NO: 230:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 70 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 230:  
 GGGAGGACGA UGCAGGCACCU UAGACCUGUC CUCCAAGCGU GAGUUGCUGU  
 GGCCCAGACG ACUCGCCCGA 50  
 70
- (2) INFORMATION FOR SEQ ID NO: 231:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 70 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 231:  
 GGGAGGACGA UGCAGGUGGUC UCCCAAUUCU AAACUUUCUC CAUCGUAUCU  
 GGCCCAGACG ACUCGCCCGA 50  
 70
- (2) INFORMATION FOR SEQ ID NO: 232:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 232:  
 GGGAGGACGA UGCAGGUCAUG GUGUCUUUCC ACAGCUCUUC CCAUGAUCGC  
 CCAGGCCAGAC GACUCGCCCG A 50  
 71
- (2) INFORMATION FOR SEQ ID NO: 233:  
 (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:

160

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 233:  
 GGGAGGACGA UGCGGGAAUJ CCCAGCGCUU GACUGAUACA AACNUUCCCG 50  
 UGCCCCAGAC GACUCGCCCG A 71
- (2) INFORMATION FOR SEQ ID NO:234:
- (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 70 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 234:  
 GGGAGGACGA UGCGGCAANN NNNNNCUCUC UCCUGGCGUU CCGCAACCCG 50  
 CCCCCAGACG ACUCGCCCG A 70
- (2) INFORMATION FOR SEQ ID NO: 235:
- (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 235:  
 GGGAGGACGA UGCGGAGUAU UCCAGCCUGG AUUCAUAGUC AGUGCUCUCC 50  
 GUGCCCAAGAC GACUCGCCCG A 71
- (2) INFORMATION FOR SEQ ID NO: 236:
- (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 236:  
 GGGAGGACGA UGCGGUCCUA GCAGCGAUUC AUCCCCGUUC UCUCAGCGUU 50  
 GCCCCCAAGAC GACUCGCCCG A 71
- (2) INFORMATION FOR SEQ ID NO: 237:
- (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:

GGGAGGACGA UGCAGGCTUGA AGUACAGGCU CUAAACUCCA AGCGCGACCG	50
UCCGCCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 238:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 238:

GGGAGGACGA UGCAGGCCCCUA CCACUUUUUC CCUCUACUGU UAUCCUGUCC	50
CCCAGACGAC UCGCCCGA	68

(2) INFORMATION FOR SEQ ID NO: 239:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 70 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 239:

GGGAGGACGA UGCAGGUGGUC UCCCCUAGAUC UACAGCACUU CCAUCGCAUU	50
GGGCCAGACG ACUCGCCCGA	70

(2) INFORMATION FOR SEQ ID NO: 240:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 240:

GGGAGGACGA UGCAGGUCAG CUUAACAGUC UGGCAAUGGC CAUUAUGGCG	50
CCCCAGACGA CUCGCCCGA	69

(2) INFORMATION FOR SEQ ID NO: 241:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid

162

(C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 241:

GGGAGGGACGA UGCGGCAGUC UGGAUCUCUA UUGGAAUUUA GUCCUCAACU	50
CUGCCCCAGA CGACUCGCCG GA	72

(2) INFORMATION FOR SEQ ID NO: 242:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 242:

GGGAGGGACGA UGCGGGAUUC UUUCGGCAAG UGAAAAAUUA CCUUGCJJCC	50
CGAGGCCAGAC GACUCGCCG A	71

(2) INFORMATION FOR SEQ ID NO: 243:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 243:

GGGAGGGACGA UGCGGGGACU UCAACUAAGU CCUCAUUUGC CUCGCUCUC	50
GUGCCCCAGAC GACUCGCCG A	71

(2) INFORMATION FOR SEQ ID NO: 244:

(i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 74 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 244:

GGGAGGGACGA UGCGGAACGG AGAUGUCCCC UCAAMAUUA CCGUCUCCGU	50
UUGCGCCCCA GACGACUCGC CCGA	74

(2) INFORMATION FOR SEQ ID NO: 245:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 70 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 245:

GGGAGGACGA UGCAGGCGAAA UUAGCUUCUU AUGACUCACG UUUCCUJUGC	50
GCCCCAGACG ACUCGCCCGA	70

(2) INFORMATION FOR SEQ ID NO: 246:

- (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 246:

GGGAGGACGA UGCAGGGCCCG AUCUACUGCA UUACCGAAAC GAUUUCCCCA	50
CUGUGCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 247:

- (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 247:

GGGAGGACGA UGCAGGGACU GAUUUUUCCU UGNCAUGUA AUUUCUGGC	50
UGCCCCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 248:

- (i) SEQUENCE CHARACTERIZATION:  
 (A) LENGTH: 71 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULAR TYPE: RNA  
 (ix) FEATURE:  
 (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
 modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 248:

GGGAGGACGA UGCAGGGACU UUGACAGGCA UUGAUUUCGA CCUGUUCCCC	50
GUGGCCAGAC GACUCGCCCG A	71

## (2) INFORMATION FOR SEQ ID NO: 249:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 249:

GGGAGGACGA UGCAGCGACA CAAUAGCCUU UGAUCCCAUG AUGGCUCGCC GUGCCCAGAC GACUCGCCCC A	50
	71

## (2) INFORMATION FOR SEQ ID NO: 250:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 250:

GGGAGGACGA UGCAGGUGUAG UUUCCUGUA UGCCAUUCUU UCCCAUGCCG CACGCCAGAC GACUCGCCCC A	50
	71

## (2) INFORMATION FOR SEQ ID NO: 251:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 251:

GGGAGGACGA UGCAGGUCGAG UGUUCUCCUU CGGUAAACUAU UNNNNAUUUC GUGCCCAGAC GACUCGCCCC A	50
	71

## (2) INFORMATION FOR SEQ ID NO: 252:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F)  
modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 252:

GGGAGGACGA UGCAGGUCGU AUUCAUCUCC UUGUUCUGUU UCGUUGCACC	50
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UGGCCAGAC GACUCGCCG A

71

## (2) INFORMATION FOR SEQ ID NO: 253:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 253:

GGGAGGACGA UGCAGGGACU UUGACAGGCA UUGAUUUCGA CGUGUUC	50
GGGCCAGAC GACUCGCCG A	71

## (2) INFORMATION FOR SEQ ID NO: 254:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 254:

GGGAGGACGA UGCAGGUGAUC AAUCGGCGCU UUACUCUUGC GCUCACCGUG	50
CCCCAGACGA CUCGCCCGA	69

## (2) INFORMATION FOR SEQ ID NO: 255:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 255:

GGGAGGACGA UGCAGGCAGUC UCCCUAGGUU UCAUCUCUGC AGCAUUCGG	50
GGUNCCAGAC GACUCGCCG A	71

## (2) INFORMATION FOR SEQ ID NO: 256:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

166

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 256:  
 GGGAGGACGA UGCGGAUCAA AAGCACUCAU UCCCGUGCUC GCUUCAUJGG               50  
 UCCCCCAGAC GACUCGCCG A    71
- (2) INFORMATION FOR SEQ ID NO: 257:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 74 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 257:  
 GGGAGGACGA UGCGGAAGAU CUCCCAACUG CUGUGGCUAA UAAUUCUCUC               50  
 CGCGUCCCCA GACGACUCGC CCGA    74
- (2) INFORMATION FOR SEQ ID NO: 258:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 70 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 258:  
 GGGAGGACGA UGCGGUCCGU CAUAACGGCC AUAAACUGCG AAUACUCCU               50  
 GGCCCAGACG ACUCGCCCGA    70
- (2) INFORMATION FOR SEQ ID NO: 259:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 73 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA
- (ix) FEATURE:
- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 259:  
 GGGAGGACGA UGCGGGGACA AWYAGCGGUG UCUUUUCAUU UNKAUCCUCC               50  
 GACRUCCAG ACGACUCGCC CGA    73
- (2) INFORMATION FOR SEQ ID NO: 260:
- (i) SEQUENCE CHARACTERIZATION:
- (A) LENGTH: 71 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 260:

GGGAGGACGA UGCGGUGACU AUCUGGCUCG AUCCAAUCAC CCGAGCCCCAC	50
CGCGCCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 261:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 261:

GGGAGGACGA UGCGGGAAACU AAUGGCCGUG AUUAACCAAU GCAGGCUUCC	50
UGCGCCAGAC GACUCGCCCG A	71

(2) INFORMATION FOR SEQ ID NO: 262:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 73 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 262:

GGGAGGACGA UGCGGUGACA UGGAAUUUUC UACGGGCCCG AUCCUGCCAG	50
CCGUGUGCAG ACGACUCGCC CGA	73

(2) INFORMATION FOR SEQ ID NO: 263:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 13 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 263:

GGAANYAANR CRR	13
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(2) INFORMATION FOR SEQ ID NO: 264:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 9 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 264:

GGAANYTGA

5

(2) INFORMATION FOR SEQ ID NO: 265:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 76 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub>, 2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: All U's are 2'NH<sub>2</sub>, 2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 265:

GGGAGACAAG AAUAACGCUC AAGUAGACUA AUGUGUGGAA GACAGCGGGU  
GGUUCGACAG GAGGCUCACA ACAGGC

50

76

(2) INFORMATION FOR SEQ ID NO: 266:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All C's are 2'-NH<sub>2</sub>, 2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: All U's are 2'NH<sub>2</sub>, 2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 266:

GGUGUGUGGAA AGACAGCGGG UGGUUC

26

(2) INFORMATION FOR SEQ ID NO: 267:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All U's and C's are 2'- deoxy-2'- aminouridine and 2'-deoxy-2' aminocytidine residues

(ix) FEATURE:

(D) OTHER INFORMATION: A at positions 14 and 17 is 2'-deoxy-2'-O-methyladenosine

(ix) FEATURE:

(D) OTHER INFORMATION: G at positions 5, 6, 22, 23, 24, 26 and 27 is 2'-deoxy-2'-O-methylguanosine

(ix) FEATURE:

169

(D) OTHER INFORMATION: T at positions 1-4 and 31-35 is 2'-deoxythymidine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:267:  
TTTTGGUGUG UGGAAGACAG CGGGUGGUUC TTTT

35

(2) INFORMATION FOR SEQ ID NO: 268:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 268:  
GGGAGGGSGSG GUYUCYYRRY UYYYYSYS

27

(2) INFORMATION FOR SEQ ID NO: 269:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 269:  
RGGRGRAYY RSBRSYYYYY BSYBSY

26

(2) INFORMATION FOR SEQ ID NO: 270:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 270:  
GGGAAGGAAY AARCRRGACC C

21

(2) INFORMATION FOR SEQ ID NO: 271:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 11 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-NH<sub>2</sub> modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 271:  
GGAAYGAYGA Y

11

170

- (2) INFORMATION FOR SEQ ID NO: 272:

  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 49 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 272:  
GGGAGGACGA UGC GGUGGUUC UCCCAAUUCU AAACUUUCUC CAUCGUAUC

49

(2) INFORMATION FOR SEQ ID NO: 273:

  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 47 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 273:  
GGGAGGACGA UGC GGUGGUUC UCCCAAUUCU AAACUUUCUC CAUCGUA

47

(2) INFORMATION FOR SEQ ID NO: 274:

  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 45 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 274:  
GGGAGGACUG CGGUGGUUC UCCAAUUCUAA ACUUUCUCCA UCGUA

45

(2) INFORMATION FOR SEQ ID NO: 275:

  - (i) SEQUENCE CHARACTERIZATION:
    - (A) LENGTH: 45 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULAR TYPE: RNA
  - (ix) FEATURE:
    - (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 275:  
GGGAGGACGA UGC GGUGGUUC UCCCCUAAAC UUUCUCCAUC GUAUC

45

(2) INFORMATION FOR SEQ ID NO: 276:

  - (i) SEQUENCE CHARACTERIZATION:

171

(A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 276:  
GGGAGGACGA UGC GGUGGUC UCCC AAUJCU AUUC UCCAUC GUAUC

45

(2) INFORMATION FOR SEQ ID NO: 277:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 277:  
GGGAGGACGA UGC GGUGGUC UCCC AAUUCU AAAC UCCAUC GUAUC

45

(2) INFORMATION FOR SEQ ID NO: 278:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 42 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 278:  
GGGAGGACGA UGC GGUGGUC UCCC AAUUA CUCAUC GUA UC

42

(2) INFORMATION FOR SEQ ID NO: 279:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 47 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 279:  
GAGGACGAUG CGGUGGUCUC CCAAUUCUAA ACUUUCUCCA UCGUAUC

47

(2) INFORMATION FOR SEQ ID NO: 280:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 42 base pairs  
(B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 280:  
GGGAGGACGA UGC GGUGGU C UCCC A UUUU CUCCAUCGU A UC 42
- (2) INFORMATION FOR SEQ ID NO: 281:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 41 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 281:  
GGGAGGACGA UGC GGUGGU C UCCC A AUUCU ACCAUCGU AU C 41
- (2) INFORMATION FOR SEQ ID NO: 282:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 44 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 282:  
GGAGGACGAU GCG GUGGU C UCC A UUCUA UUCUCCAUCG UAUC 44
- (2) INFORMATION FOR SEQ ID NO: 283:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 283:  
GGAGGACGAU GCG GUGGU C UCC A UUCUA UCUCCAUCG U AUC 43
- (2) INFORMATION FOR SEQ ID NO: 284:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- (ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 284:  
GAGGACGAUG CGGUGGGUCUC CCAAUUCUAU UCUCCAUCGU AUC 43
- (2) INFORMATION FOR SEQ ID NO: 285:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 41 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 285:  
GAGGACGAUG CGGUGGGUCUC AAUUCUAUUC UCCAUUCGUAU C 41
- (2) INFORMATION FOR SEQ ID NO: 286:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 44 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 286:  
GGGAGGCGAU GCGGUGGGUCU CCCAAUUCUA UUCUCCAUCG UAUC 44
- (2) INFORMATION FOR SEQ ID NO: 287:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 44 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 287:  
GGGAGGACGA UGCUGGGUGUC UCCAAUUCU AUCUCCAUCG UAUC 44
- (2) INFORMATION FOR SEQ ID NO: 288:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:

174

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 288:  
GGGAGGACGA UGC GGUGGU C UCCC AAUUCU ACUCC AU CGU AUC

43

(2) INFORMATION FOR SEQ ID NO: 289:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 289:  
GGGAGGACGA UGC GGUGGU C UCCC AAUUCU AU CC AU CGUA UC

42

(2) INFORMATION FOR SEQ ID NO: 290:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 44 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 290:  
GGGAGGACGA UGC GGUGGU C UCCC AAUUCU UUCU CC AU CG UA UC

44

(2) INFORMATION FOR SEQ ID NO: 291:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 43 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 291:  
GGGAGGACGA UGC GGUGGU C UCCC AAUUCU UC UCC AU CGU AUC

43

(2) INFORMATION FOR SEQ ID NO: 292:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(ix) FEATURE:

(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 292:  
GGGGACGAUG CGGUGGCCAA AUUCUAUUC UCCAUCGUAU C

41

## (2) INFORMATION FOR SEQ ID NO: 293:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 293:  
GGGACCGAUGC GGUGGCCAAU UUCUAUUCUC CAUCGUAUC

39

## (2) INFORMATION FOR SEQ ID NO: 294:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 294:  
GACGAUGCGG UGGCCCAAUU CUUAUUCUCC AUCGUAUC

37

## (2) INFORMATION FOR SEQ ID NO: 295:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 295:  
GGACGAUGCG GUGGCCAAU UCUAUUCUCC AUCGUAUC

38

## (2) INFORMATION FOR SEQ ID NO: 296:

## (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULAR TYPE: RNA

## (ix) FEATURE:

- (D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F)  
modified

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 296:  
GAUGCGGUGG CCCAAUUCUA UUCUCCAUCG UAUC

34

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(2) INFORMATION FOR SEQ ID NO: 297:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 37 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 297:  
GAUGCAGGG UCUCCCAAUU CUAUUCUCCA UCGUAUC

37

(2) INFORMATION FOR SEQ ID NO: 298:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 298:  
GGUGGUCUCC CAAUUCUAUU CUCCAUCGUA UC

32

(2) INFORMATION FOR SEQ ID NO: 299:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 12 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 299:  
GGGAGGACGA UG

12

(2) INFORMATION FOR SEQ ID NO: 300:  
(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 42 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 300:  
GGGAGGACGA UGCAGGGUC UCCCAAUUCU CUCCAUCGUA UC

42

(2) INFORMATION FOR SEQ ID NO: 301:  
(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 41 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 301:

GGGAGGACGA UGC GGUGUCU CCCAUUCUU CUCAUCGUAU C

41

(2) INFORMATION FOR SEQ ID NO: 302:

(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 41 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 302:

GGGAGGACGA UGC GGGGUCU CCCAUUCUU CUCCUCGUAU C

41

(2) INFORMATION FOR SEQ ID NO: 303:

(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 39 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 303:

GGGAGGACGA UGC GGGUCUC CCAUUCUU UCUCGUAU C

39

(2) INFORMATION FOR SEQ ID NO: 304:

(i) SEQUENCE CHARACTERIZATION:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULAR TYPE: RNA  
(ix) FEATURE:  
(D) OTHER INFORMATION: All pyrimidines are 2'-fluoro (2'-F) modified  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 304:

GGGAGUACGA UGC CGGUGGUUC UCCAAUUCU UCUCCAUCGU AUC

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**WE CLAIM:**

1. A method of identifying nucleic acid ligands to TGF $\beta$ , comprising:
  - a) contacting a candidate mixture of nucleic acids with TGF $\beta$ , wherein nucleic acids having an increased affinity to TGF $\beta$  relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and
  - b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
  - c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to TGF $\beta$ , whereby nucleic acid ligands of TGF $\beta$  may be identified.
2. The method of claim 1 further comprising:
  - (d) repeating steps a), b), and c).
3. The method of claim 1 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.
4. The method of claim 3 wherein said single stranded nucleic acids are ribonucleic acids.
5. The method of claim 4 wherein said nucleic acids comprise modified nucleic acids.
6. The method of claim 5 wherein said nucleic acids are 2'-amino (2'-NH<sub>2</sub>) modified ribonucleic acids.
7. The method of claim 5 wherein said nucleic acids are 2'-F modified ribonucleic acids.
8. The method of claim 5 wherein said nucleic acids are 2'-NH<sub>2</sub>-UTP, 2'-F-CTP modified ribonucleic acids.
9. The method of claim 5 wherein said nucleic acids are 2'-F-UTP, 2'-NH<sub>2</sub>-CTP modified ribonucleic acids.
10. The method of claim 3 wherein said single stranded nucleic acids are deoxyribonucleic acids.

11. A method for treating TGF $\beta$ -mediated pathological conditions comprising administering a pharmaceutically effective amount of a TGF $\beta$  nucleic acid ligand.

5 12. The method of claim 11 wherein said TGF $\beta$  nucleic acid ligand is identified according to the method of claim 1.

13. A method for treating TGF $\beta$ 1-mediated pathological conditions comprising administering a pharmaceutically effective amount of a TGF $\beta$ 1 nucleic acid ligand.

10 14. The method of claim 13 wherein said TGF $\beta$ 1 nucleic acid ligand is identified according to the method of claim 1.

15. The method of claim 14 wherein said ligand is selected from the ligands of Table 3 and 6 (SEQ ID NOS:12-42; 55-89).

16. A purified and isolated non-naturally occurring nucleic acid ligand to TGF $\beta$ .

15 17. A purified and isolated non-naturally occurring nucleic acid ligand to TGF $\beta$ 1.

18. The purified and isolated non-naturally occurring nucleic acid ligand of claim 17 wherein said nucleic acid ligand is single-stranded.

20 19. The purified and isolated non-naturally occurring ligand of claim 18 wherein said nucleic acid ligand is ribonucleic acid.

20. The purified and isolated non-naturally occurring ligand of claim 18 wherein said nucleic acid ligand is deoxyribonucleic acid.

21. A nucleic acid ligand to TGF $\beta$  identified according to the method comprising:

25 a) contacting a candidate mixture of nucleic acids with TGF $\beta$ , wherein nucleic acids having an increased affinity to TGF $\beta$  relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

30 b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to TGF $\beta$ , whereby nucleic acid ligands of TGF $\beta$  may be identified.

5        22. A nucleic acid ligand to TGF $\beta$ 1 identified according to the method comprising:

a) contacting a candidate mixture of nucleic acids with TGF $\beta$ 1, wherein nucleic acids having an increased affinity to TGF $\beta$ 1 relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to TGF $\beta$ 1, whereby nucleic acid ligands of TGF $\beta$ 1 may be identified.

10        23. The purified and isolated non-naturally occurring ribonucleic acid ligand to TGF $\beta$ 1 of claim 19 wherein said ligand is selected from the group consisting of the sequences set forth in Table 3 (SEQ ID NOS:12-42).

15        24. The purified and isolated non-naturally occurring ribonucleic acid ligand to TGF $\beta$ 1 of claim 19 wherein said ligand is substantially homologous to and has substantially the same ability to bind TGF $\beta$ 1 as a ligand selected from the group consisting of the sequences set forth in Table 3 (SEQ ID NOS:12-42).

20        25. The purified and isolated non-naturally occurring ribonucleic acid ligand to TGF $\beta$ 1 of claim 19 wherein said ligand has substantially the same structure and substantially the same ability to bind TGF $\beta$ 1 as a ligand selected from the group consisting of the sequences set forth in Table 3 (SEQ ID NOS:12-42).

26. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to TGF $\beta$ 1 of claim 20 wherein said ligand is selected from the group consisting of the sequences set forth in Table 6 (SEQ ID NOS:55-89).

5        27. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to TGF $\beta$ 1 of claim 20 wherein said ligand is substantially homologous to and has substantially the same ability to bind TGF $\beta$ 1 as a ligand selected from the group consisting of the sequences set forth in Table 6 (SEQ ID NOS:55-89).

10        28. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to TGF $\beta$ 1 of claim 20 wherein said ligand has substantially the same structure and substantially the same ability to bind TGF $\beta$ 1 as a ligand selected from the group consisting of the sequences set forth in Table 6 (SEQ ID NOS:55-89).

29. A method of identifying nucleic acid ligands of PDGF, comprising:

15        a) contacting a candidate mixture of nucleic acids with PDGF, wherein nucleic acids having an increased affinity to PDGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

20        b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

25        c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to PDGF, whereby nucleic acid ligands of PDGF may be identified.

30. The method of claim 29 further comprising:

25        d) repeating steps a), b), and c).

31. The method of claim 29 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.

32. The method of claim 31 wherein said single stranded nucleic acids are ribonucleic acids.

33. The method of claim 31 wherein said single stranded nucleic acids are deoxyribonucleic acids.

34. The method of claim 32 wherein said nucleic acids comprise modified nucleic acids.

5       35. The method of claim 34 wherein said nucleic acids are 2'-amino (2'-NH<sub>2</sub>) modified ribonucleic acids.

36. The method of claim 34 wherein said nucleic acids are 2'-fluoro (2'-F) modified ribonucleic acids.

10      37. A method for treating a PDGF-mediated disease comprising administering a pharmaceutically effective amount of a nucleic acid ligand of PDGF.

38. The method of claim 37 wherein said nucleic acid ligand is identified according to the method of claim 29.

15      39. The method of claim 38 wherein said nucleic acid ligand is selected from one of the ligands of Tables 8 and 13, and Figures 3, 4, and 10 (SEQ ID NOS.93-124, 128-176).

40. A purified and isolated non-naturally occurring nucleic acid ligand to PDGF.

20      41. The purified and isolated non-naturally occurring nucleic acid ligand of claim 40 wherein said nucleic acid ligand is single-stranded.

42. The purified and isolated non-naturally occurring nucleic acid ligand of claim 41 wherein said nucleic acid ligand is ribonucleic acid.

43. The purified and isolated non-naturally occurring nucleic acid ligand of claim 41 wherein said nucleic acid ligand is deoxyribonucleic acid.

25      44. A nucleic acid ligand to PDGF identified according to the method comprising:

30      a) contacting a candidate mixture of nucleic acids with PDGF, wherein nucleic acids having an increased affinity to PDGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

- b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
- 5 c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to PDGF, whereby nucleic acid ligands of PDGF may be identified.

45. The purified and isolated non-naturally occurring ribonucleic acid ligand to PDGF of claim 42 wherein said ligand is selected from the group consisting of the sequences set forth in Table 13 (SEQ ID NOS:128-170).

10 46. The purified and isolated non-naturally occurring ribonucleic acid ligand to PDGF of claim 42 wherein said ligand is substantially homologous to and has substantially the same ability to bind PDGF as a ligand selected from the group consisting of the sequences set forth in Table 13 (SEQ ID NOS:128-170).

15 47. The purified and isolated non-naturally occurring ribonucleic acid ligand to PDGF of claim 42 wherein said ligand has substantially the same structure and substantially the same ability to bind PDGF as a ligand selected from the group consisting of the sequences set forth in Table 13 (SEQ ID NOS:128-170).

20 48. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to PDGF of claim 43 wherein said ligand is selected from the group consisting of the sequences set forth in Tables 8 and 9, and Figures 3, 4, and 10 (SEQ ID NOS:93-124, 171-176).

25 49. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to PDGF of claim 43 wherein said ligand is substantially homologous to and has substantially the same ability to bind PDGF as a ligand selected from the group consisting of the sequences set forth in Tables 8 and 9, and Figures 3, 4 and 10 (SEQ ID NOS:93-124, 171-176).

30 50. The purified and isolated non-naturally occurring deoxyribonucleic acid ligand to PDGF of claim 43 wherein said ligand has substantially the same structure and substantially the same ability to bind PDGF as a ligand selected from

the group consisting of the sequences set forth in Tables 8 and 9, and Figures 3, 4 and 10 (SEQ ID NOS:93-124, 171-176).

5        51. The purified and isolated non-naturally occurring nucleic acid ligand to PDGF of claim 40 comprising the conserved structure shown in Figure 3 (SEQ ID NO:171).

10        52. A method of identifying nucleic acid ligands to hKGF, comprising:

a)        contacting a candidate mixture of nucleic acids with hKGF, wherein nucleic acids having an increased affinity to hKGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b)        partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c)        amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to hKGF, whereby nucleic acid ligands of hKGF may be identified.

15        53. The method of claim 52 further comprising:

d)        repeating steps a), b), and c).

20        54. The method of claim 52 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.

55. The method of claim 54 wherein said single stranded nucleic acids are ribonucleic acids.

25        56. The method of claim 55 wherein said nucleic acids comprise modified nucleic acids.

57. The method of claim 56 wherein said nucleic acids are 2'-amino (2'-NH<sub>2</sub>) modified ribonucleic acids.

25        58. The method of claim 56 wherein said nucleic acids are 2'-F modified ribonucleic acids.

59. A method for treating hKGF-mediated pathological conditions comprising administering a pharmaceutically effective amount of a hKGF nucleic acid ligand.

60. The method of claim 59 wherein said hKGF nucleic acid ligand is identified according to the method of claim 52.

61. The method of claim 60 wherein said ligand is selected from one of the ligands of Tables 16 and 23 (SEQ ID NOS:189-262, 272-304).

62. A method of assaying a test compound for the ability to inhibit hKGF receptor-mediated cell proliferation, the method comprising the steps of:

- 10 a) contacting the test compound with a hKGF nucleic acid ligand and a keratinocyte growth factor; and  
b) detecting the ability of the test compound to inhibit binding between the hKGF nucleic acid ligand and the keratinocyte growth factor.

63. A method for assaying a test compound for the ability to inhibit the interaction of a growth factor with its plasma membrane bound receptor, said method comprising the steps of:

- 15 a) solubilizing cells containing said plasma membrane bound receptor;  
b) creating a plasma membrane extract of said cells;  
c) reacting said extract with labeled growth factor alone and in the presence of the test compound thereby creating complexes;  
20 d) analyzing said complexes by electrophoresis under native conditions;  
e) visualizing said complexes by imaging; and  
25 e) comparing the image of said extract with labeled growth factor alone to the image of said extract in the presence of the test compound to determine whether said test compound inhibited the interaction between said growth factor and its plasma membrane bound receptor.

64. The method of claim 63 wherein said growth factor is hKGF.

30 65. The method of claim 63 wherein said cells are PC-3 cells.

66. The method of claim 63 wherein said test compound is selected from the group consisting of a small molecule, a peptide, and an antibody.

67. The method of claim 63 wherein said imaging is selected from the group consisting of autoradiography and phosphorimaging.

5       68. A method for assaying cells to determine whether they express a growth factor plasma membrane bound receptor, said method comprising the steps of:

a) solubilizing said cells;

b) creating a plasma membrane extract of said cells;

10       c) reacting said plasma membrane extract with a labeled growth factor;

d) analyzing the reaction between said plasma membrane extract with said labeled growth factor by electrophoresis under native conditions;

15       e) comparing the electrophoresis of step d) with electrophoresis of labeled growth factor; and

f) visualizing the results of the electrophoresis to determine whether a complex is formed with altered mobility relative to the mobility of a labeled growth factor alone.

69. A purified and isolated non-naturally occurring nucleic acid ligand to  
20 hKGF.

70. The purified and isolated non-naturally occurring nucleic acid ligand of claim 69 wherein said nucleic acid is single stranded.

71. The purified and isolated non-naturally occurring nucleic acid ligand of claim 70 wherein said nucleic acid is ribonucleic acid.

25       72. A nucleic acid ligand to hKGF identified according to the method comprising:

a) contacting a candidate mixture of nucleic acids with hKGF, wherein nucleic acids having an increased affinity to hKGF relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

- b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
- 5 c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to hKGF, whereby nucleic acid ligands of HKGF may be identified.

73. The purified and isolated non-naturally occurring ribonucleic acid ligand to hKGF of claim 71 wherein said ligand is selected from the group consisting of the sequences set forth in Tables 16 and 23 (SEQ ID NOS:189-262, 10 272-304).

15 74. The purified and isolated non-naturally occurring ribonucleic acid ligand to hKGF of claim 71 wherein said ligand is substantially homologous to and has substantially the same ability to bind hKGF as a ligand selected from the group consisting of the sequences set forth in Tables 16 and 23 (SEQ ID NOS:189-262, 272-304).

20 75. The purified and isolated non-naturally occurring ribonucleic acid ligand to hKGF of claim 71 wherein said ligand has substantially the same structure and substantially the same ability to bind hKGF as a ligand selected from the group consisting of the sequences set forth in Tables 16 and 23 (SEQ ID NOS:189-262, 272-304).

76. A purified and isolated non-naturally occurring ribonucleic acid ligand to bFGF wherein said ligand has the sequence as shown in SEQ ID NO:267.

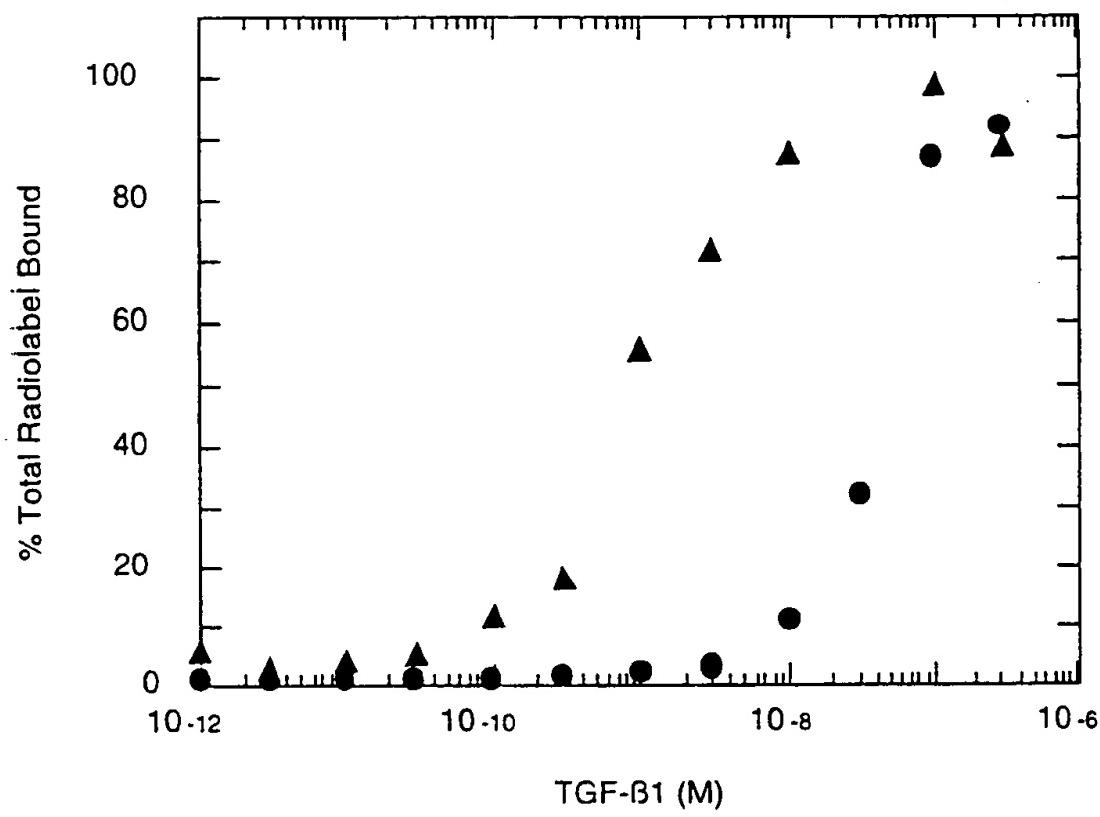


FIGURE 1

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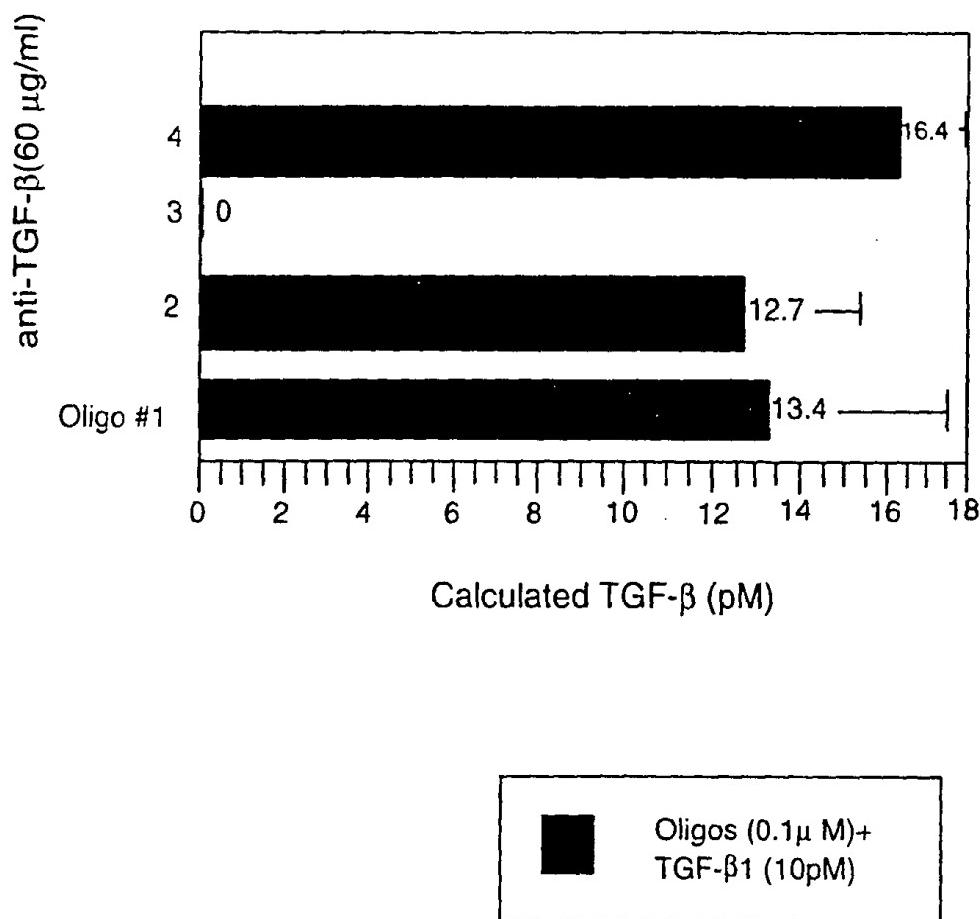
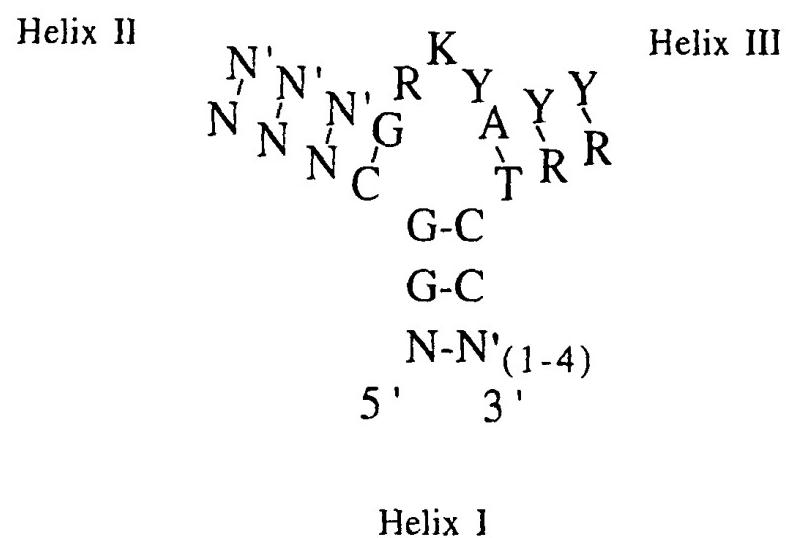


FIGURE 2

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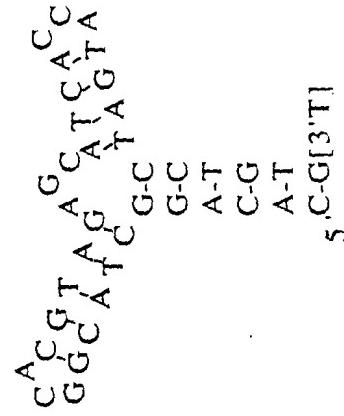


SEQ ID NO: 171

FIGURE 3

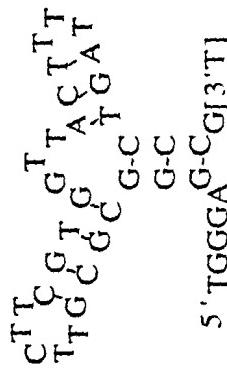
4/17

36t



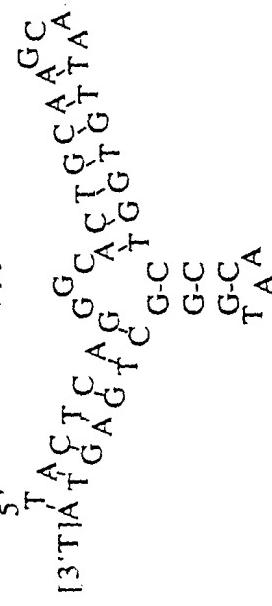
SEQ ID NO: 173

20t



SEQ ID NO: 172

41t



SEQ ID NO: 174

FIGURE 4

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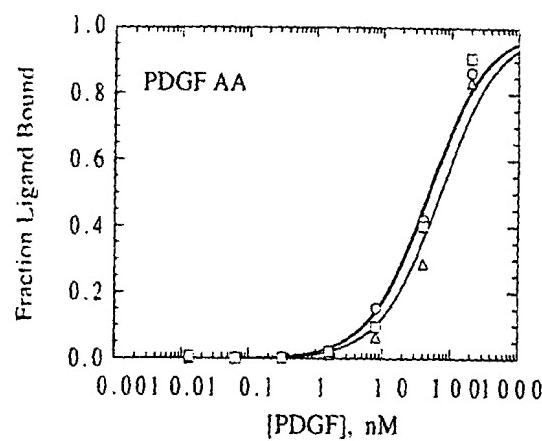


Figure 5A

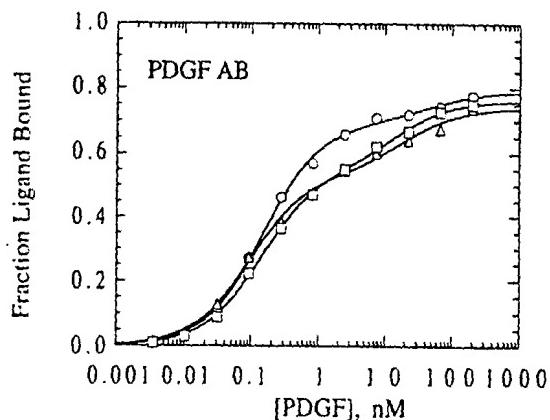


Figure 5B

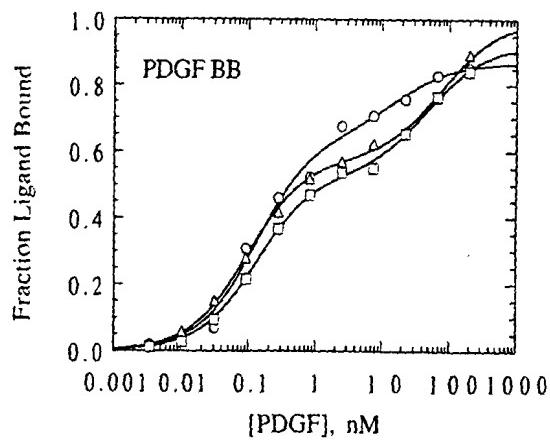


Figure 5C  
SUBSTITUTE SHEET (RULE 26)

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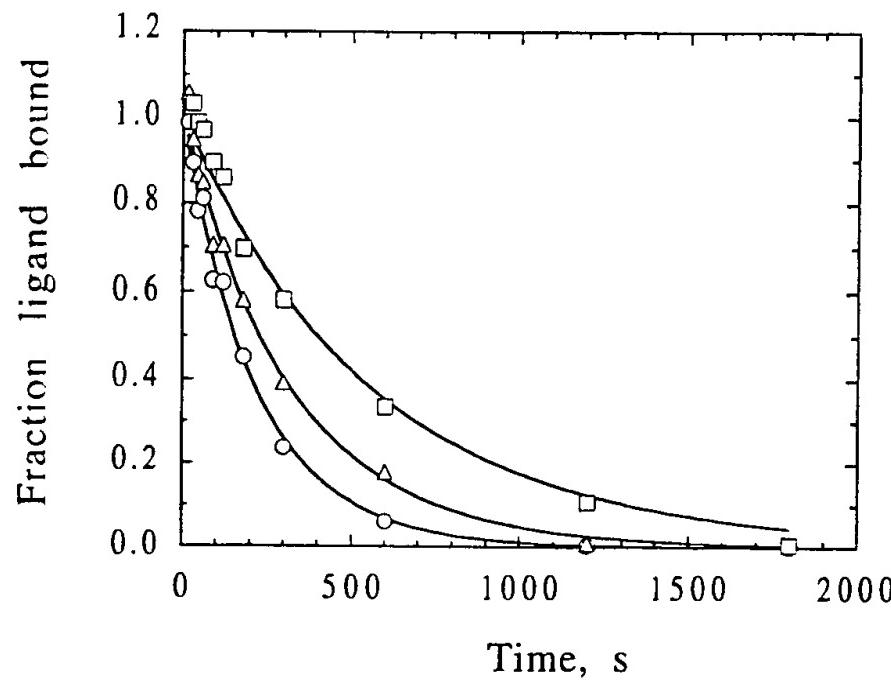


FIGURE 6

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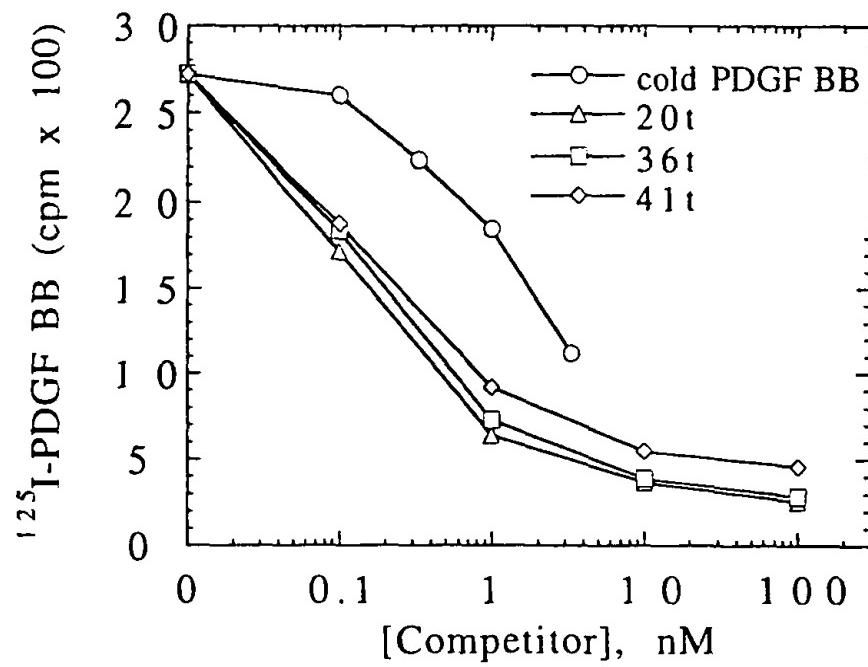


FIGURE 7

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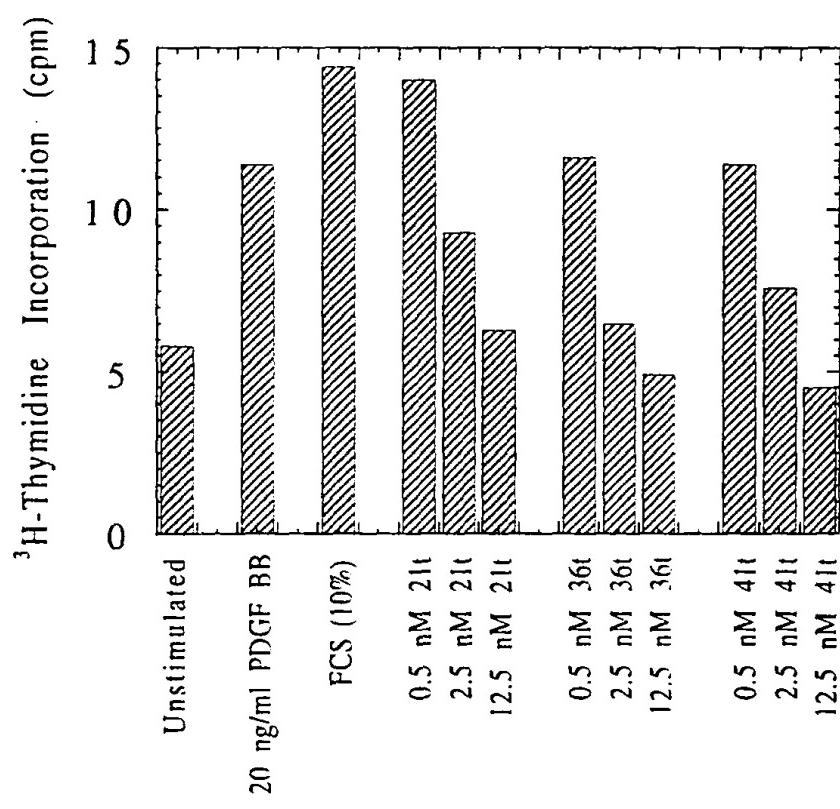
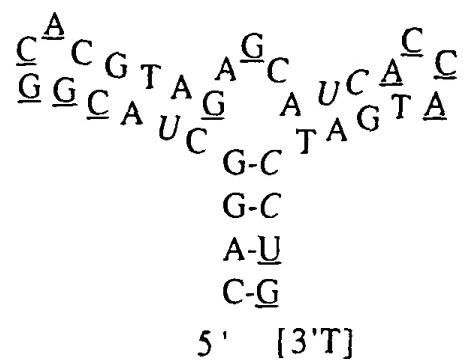
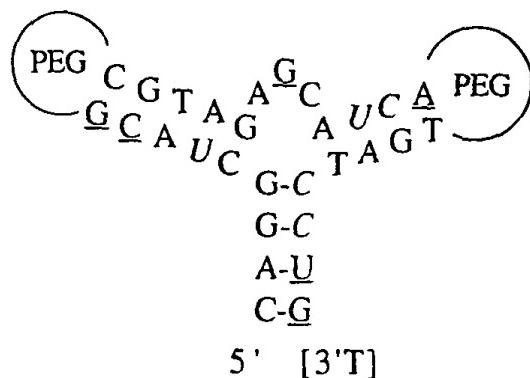


FIGURE 8

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 $K_d = 0.065 \text{ nM}$ 

SEQ ID NO: 175

 $K_d = 0.097 \text{ nM}$ 

SEQ ID NO: 176

FIGURE 9

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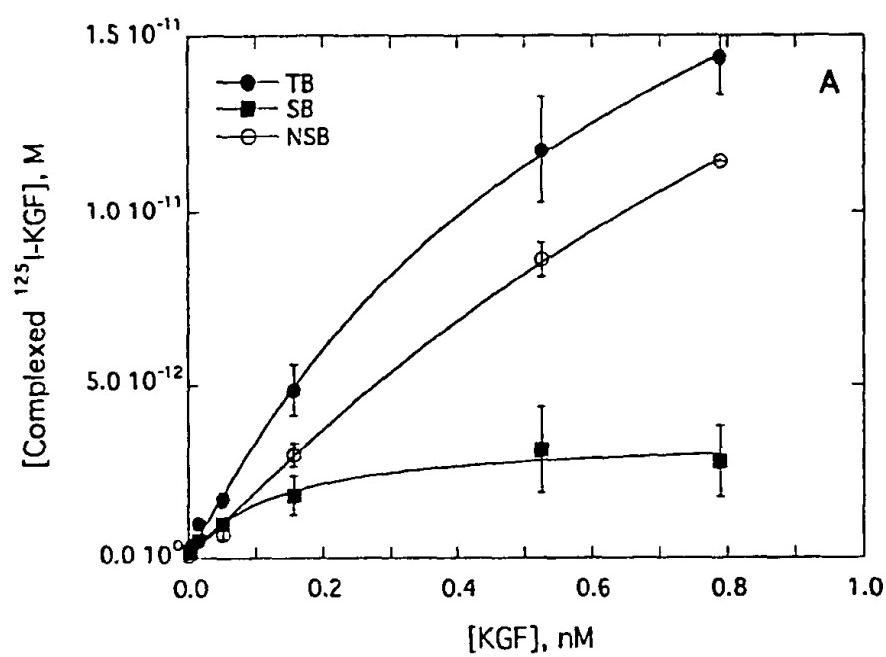


FIGURE 10A

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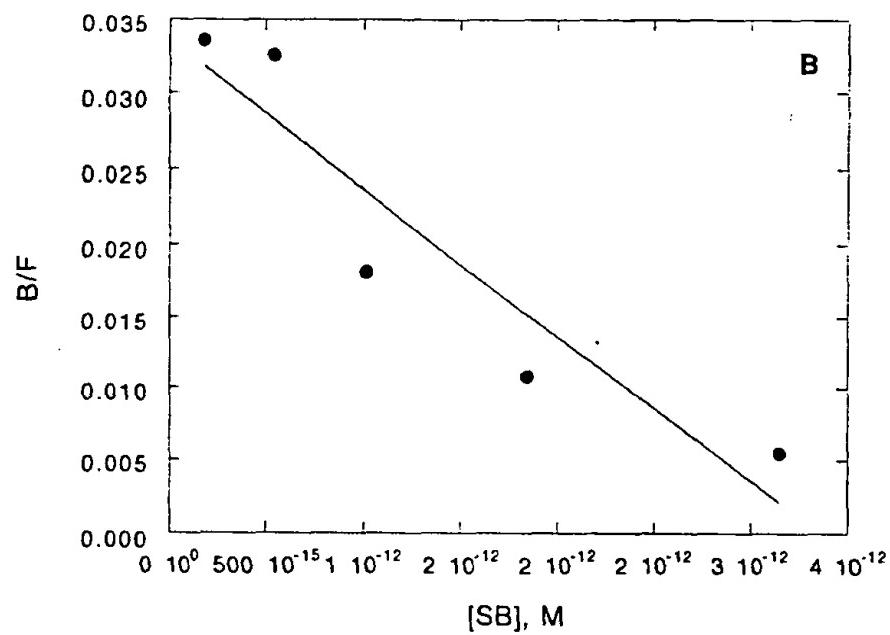


FIGURE 10B

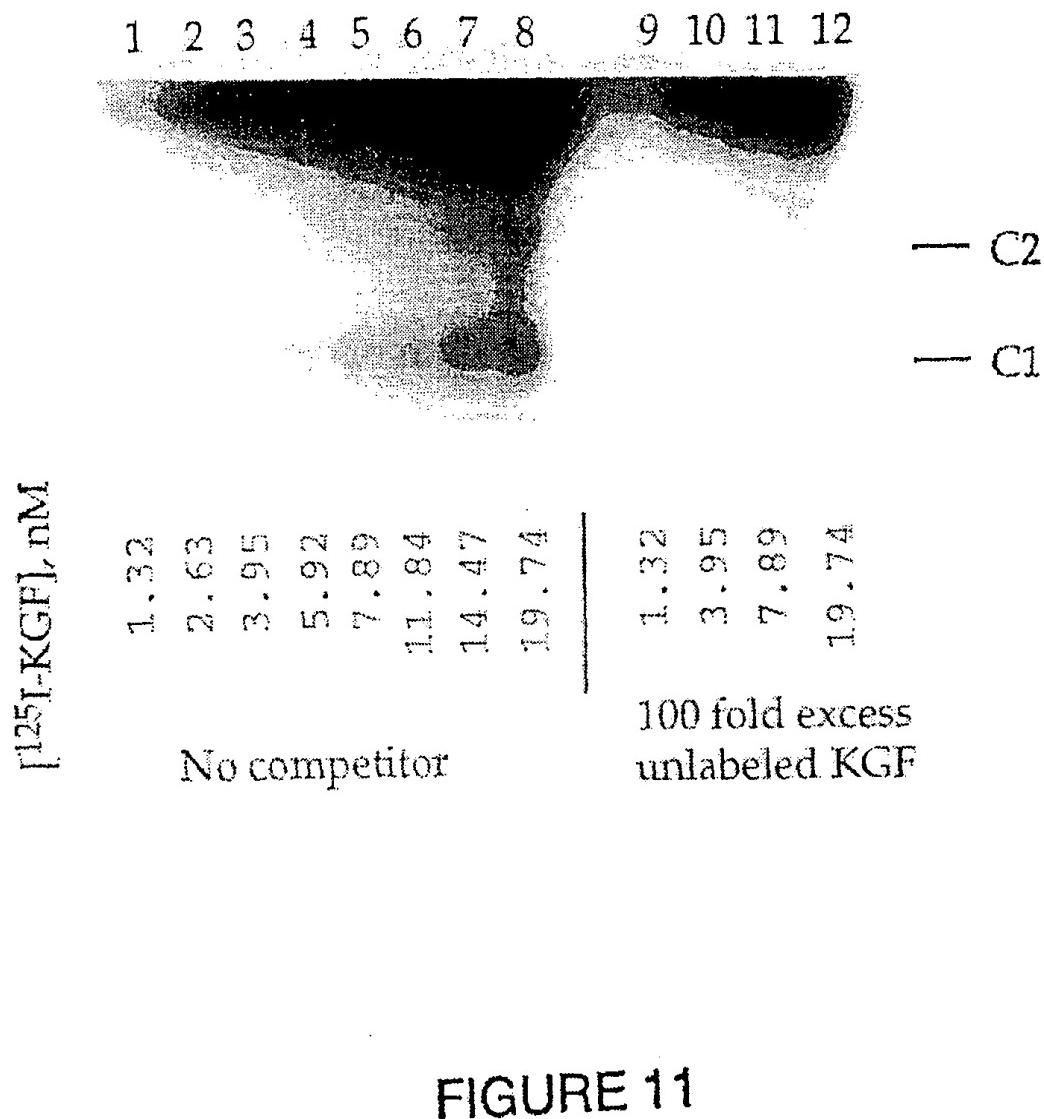


FIGURE 11

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class 1

Summary Structure

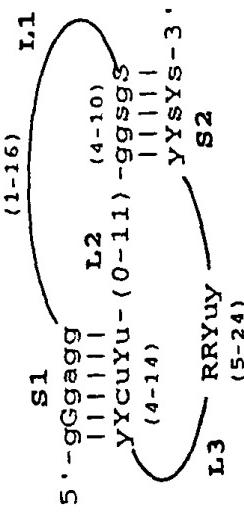


FIGURE 12A

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Class 2		Kd, nM	Ki, nM	
SEQ ID NO:	Clone			
230	*13F	0.03	10.0	ggacgauccaa cgauccaa
254	*50F	0.12		ggatcaaa
247	38F	0.33	0.2	ggatcaaa
227	10F	0.47	10.0	ggatcaaa
226	9F	0.83		ggatcaaa
256	53F	0.7		ggatcaaa
253	49F	1.0		ggatcaaa
250	43F	1.13	16.7	ggatcaaa
260	57P	1.2		ggatcaaa
248	41F	1.44		ggatcaaa
241	27F	1.52		ggatcaaa
235	20F	2.05		ggatcaaa
258	55F	2.52		ggatcaaa
261	58F	2.52		ggatcaaa
221	3F	4.47		ggatcaaa
236	21F	ND		ggatcaaa
242	28F	ND		ggatcaaa
	Consensus	2.69		rgrrg

CACCUAGACCUGUCUCCAAACGGUGAUUG  
GGGGGU  
NCAGCUC  
UGGGGA  
ACUAGG  
AGCACUA  
UUGACAC  
augccggauAG  
GGCCGGAU  
GACAGG  
UGGAAUC  
AUUAGGCCAU  
CGACCCCACCG  
CCUGUCCCCG  
AUUAGGCCAU  
UGCUCCCCGGCCcaga  
UGACAUUC  
cgggCACUU  
cgGAGIC  
AGUCAG  
GGCCAUAAAUCGGAAUA  
UGGCGGUAUAACCAAGCAAG  
GCCACUCA  
GCAGCGAUCAUCC  
UCCIU  
GCTUCCGGAGC  
syRy

## Summary Structure

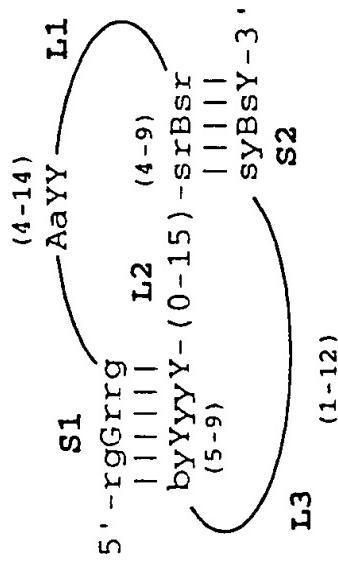


FIGURE 12B

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Class 1		Kd, nM	Kd, nM	Kd, nM	Kd, nM
SEQ ID NO:	Clone	14N	0.4	2.6	0.4
195	UCCAGGGAU	<u>UGAAGUGUCGGGGGUAGGA</u>	<u>GGAA</u>	<u>AGAA</u>	<u>A</u>
199	<u>augccgca</u>	<u>GGCAAU</u>	<u>GCGAUG</u>	<u>AAGGGG</u>	<u>UAGC</u>
191	<u>augccggcg</u>	<u>GGGGGG</u>	<u>AGGGGG</u>	<u>UACGGG</u>	<u>ACGG</u>
189	<u>GGAAAGG</u>	<u>ACGAUAAA</u>	<u>AGGA</u>	<u>UCGAA</u>	<u>UAGC</u>
205	<u>GGGA</u>	<u>GGGGGGGGGG</u>	<u>AGGGGAA</u>	<u>GGAA</u>	<u>GGAA</u>
203	<u>augccggccg</u>	<u>AGAGCCU</u>	<u>ANUGGA</u>	<u>GUCCAU</u>	<u>CAGUC</u>
207	<u>augccggcg</u>	<u>ACCU</u>	<u>AGACGGGAA</u>	<u>ACAUAGAACATICAAC</u>	<u>GCGGCAG</u>
196	<u>CGAAGCGGAAGGUUCA</u>	<u>ACAUAGUAGGGAA</u>	<u>GGUUC</u>	<u>CA CU</u>	<u>UGAA</u>
	<u>GGG</u>	<u>aa</u>	<u>GGAA</u>	<u>YAA</u>	<u>RcRR</u>
Consensus					

### Summary Structure

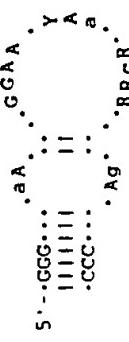


FIGURE 12C

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class 2

SUMMARY STATEMENT

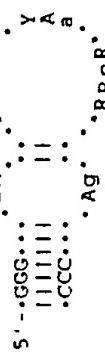


FIGURE 12D

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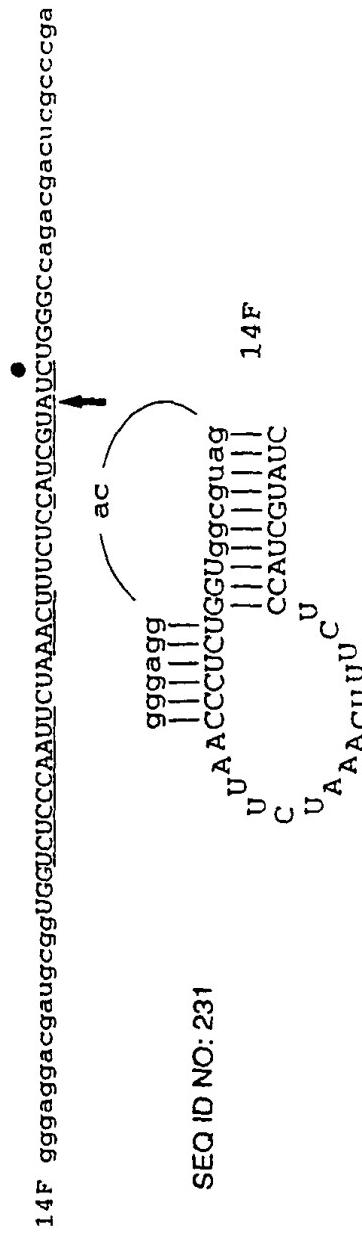
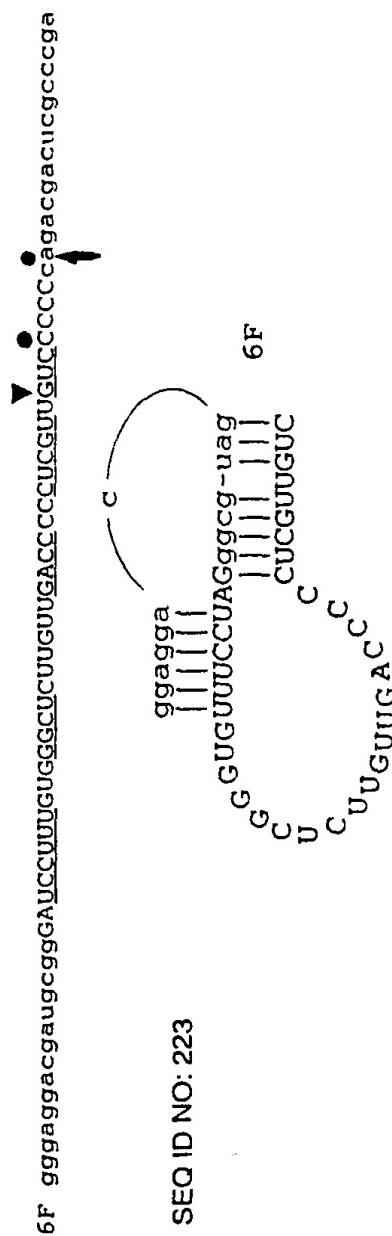


FIGURE 13

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/08014

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12P 19/34; C12Q 1/68  
 US CL :435/6, 92.1; 935/77, 78

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 92.1; 935/77, 78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

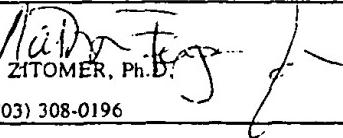
APS, DIALOG ONESEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,270,163 A (L. GOLD ET AL.) 14 December 1993 (14.12.93), columns 49-51.	1-10, 29-36, 52-58
Y, P	US 5,459,015 A (N. JANJIC ET AL.) 17 October 1995 (17.10.95), see entire document, especially columns 13-17.	1-10, 29-36, 52-58

<input type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
*A*	Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*E*	document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L*	earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O*	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*&*	document member of the same patent family
*P*	document referring to an oral disclosure, use, exhibition or other means		
	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
12 AUGUST 1996	27 AUG 1996

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer STEPHANIE W. ZITOMER, Ph.D.  Telephone No. (703) 308-0196
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Form PCT/ISA/210 (second sheet)(July 1992)\*

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08014

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-10, 29-36 and 52-58

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08014

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-10, 29-36 and 52-58, drawn to a method for identifying nucleic acid ligands to a growth factor;

Group II, claims 11-15, 37-39 and 59-61, drawn to a method for treating growth factor-mediated pathological conditions;

Group III, claims 16-28, 40-51 and 69-76, drawn to nucleic acid ligands to growth factors;

Group IV, claims 62-68, drawn to an assay for a test compound or growth factor receptor.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature linking the invention Groups is the growth factors. However, the claimed growth factors, TGF $\beta$ , PDGF and hKGF, do not make a contribution over the prior art because they were known (see citations at pages 1-13 of the description) and the Group I method does not make a contribution over the prior art because the prior art teaches the method with growth factor as target (Gold et al., US 5,270,163, Example 10, columns 49-51). Therefore, lack of unity is present.

1.